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THE UNIVERSITY OF ALBERTA

GALLIUM-68 POLYPHOSPHATE: A NEW RADIOPHARMACEUTICAL

by

© RICHARD T. GOULET

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Gallium-68 Polyphosphate: A New Radiopharmaceutical" submitted by Richard T. Goulet in partial fulfilment of the requirements for the degree of Master of Science in Radiopharmacy.

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ABSTRACT

Various ^{68}Ga radiopharmaceuticals were investigated for their potential use as bone scanning agents.

A method was developed for the preparation of ^{68}Ga-polyphosphate, ^{68}Ga-gallium-polyphosphate and $^{68}Ga(OH)_3$. Animal distribution studies indicated that six hours after the intravenous administration of ^{68}Ga-polyphosphate to mice, approximately 50% of the administered dose accumulated in the bone, with about 16% remaining in the blood. The addition of carrier gallium to the ^{68}Ga-polyphosphate complex (^{68}Ga-gallium-polyphosphate) enhanced the bone uptake of the complex. Bone levels four hours after the injection reached approximately 64% of the administered dose while the blood level was only 6%. The tissue-to-blood ratio calculated for the ^{68}Ga-gallium-polyphosphate indicated that bone was the only tissue which actively concentrated the complex. After the intravenous administration of ^{68}Ga-gallium-polyphosphate to rabbits, the complex accumulated in the bone mineral by a factor of 20 times greater than in the bone marrow.

Approximately 35% of the administered radioactivity of $^{68}Ga(OH)_3$ localized in the liver within four hours following the intravenous administration to mice. This suggested the presence of colloidal particles in the preparation.

Toxicity studies indicated that sodium tripolyphosphate, at an intravenous dose of 200 mg/kg in mice, was acutely toxic. Sodium tripolyphosphate doses below this level and containing carrier gallium were non-toxic. No histopathological changes were apparent at any of the dose levels investigated in any of the tissues examined.

Bone images were obtained on a Pho/Gamma Positron III Scintillation Camera following the intravenous administration of ^{68}Ga-polyphosphate or ^{68}Ga-gallium-polyphosphate to rabbits.

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INTRODUCTION

The use of short-lived radioisotopes for imaging procedures in nuclear medicine has become increasingly popular (1). With the advent of such radioisotope generators as the ^{99}Mo-^{99m}Tc, ^{113}Sn-^{113m}In and ^{68}Ge-^{68}Ga systems, a convenient and practical source of the short-lived radioisotopes has been established. These generator systems yield radioisotopes possessing a high degree of purity, both chemical and radionuclidic (2).

Recent investigations using ^{99m}Tc-labelled sodium tripolyphosphate in the field of bone imaging (3) have motivated additional research in this area to design a radiopharmaceutical that would possess the following desired characteristics:

(i) the radioisotope selected should be of sufficient short half-life to warrant its practical use

(ii) it should be available, if possible, through a radioisotope generator of sufficient long-life

(iii) a significant fraction of the injected material should be concentrated rapidly by the bone, therefore the agent must have a strong affinity for bone

(iv) the bone scanning agent must possess a differential uptake between tumor-involved and normal bone

(v) a high bone-to-background ratio is advantageous; this would necessitate rapid clearing of the agent from the soft tissues and blood and rapid renal excretion of the fraction not accumulated by bone

(vi) it should be possible to administer millicurie amounts of radioactivity resulting in a shorter scanning time with an increase in resolution

(vii) it is desirable that scanning or imaging procedures be started as soon as possible following the injection of the radiopharmaceutical.

Since both gallium and polyphosphates are known to possess an affinity toward bone, it seemed logical to investigate a preparation incorporating the characteristics of both these compounds. The parameters studied included the chemical preparation of the complex, its tissue distribution and its potential toxicity.

The radioisotope generator which incorporated the parent-daughter pair ^{68}Ge-^{68}Ga was first proposed by Gleason (4) and later modified by Green (5) and Yano (6). Gallium-68 decays mainly by positron emission, permitting the application of coincidence detection techniques with a positron camera. Gallium-68 radiopharmaceuticals have been used in imaging procedures for various organs including the brain, bone, bone marrow, lung, kidney and liver. Gallium-68 was chosen for this study on the basis of its

one hour half-life, its availability from a generator system and on the possibility of using coincidence detection techniques allowing the administration of lower amounts of radioactivity as compared to some agents currently used for bone scanning.

SURVEY OF THE LITERATURE

I. Gallium

A. History and Chemistry of Gallium

Gallium, with an atomic number of 31 and an atomic weight of 69.72, belongs to group three of the periodic table (7). The existence of gallium had been predicted by the Russian chemist D.I. Mendeleev who in 1871 described the unknown element as eca aluminum. Lecoq de Boisbaudran, a French spectroscopist, was credited with the actual discovery of gallium in 1875 from his observation of two intense lines in the spark spectrum of sphalerite, with wavelengths of 4172 and 4033Å, which he ascribed to the new element (7).

Gallium is closely associated with aluminum and is found in ores and minerals containing aluminum. They have similar chemical properties as well as having a similarity in the structure of the outermost shells of their atoms. Both have the same charge on their ions, (Ga^{3+}, Al^{3+}) (7).

In nature gallium exists as a mixture of two stable isotopes ^{69}Ga (60.5%) and ^{71}Ga (39.5%) (7). In addition, many artificial radioisotopes of gallium are known to exist (7,8,9).

Anhydrous gallium trichloride, $GaCl_3$, is very hygroscopic. It fumes in air as it absorbs moisture and is converted into a gel-like substance (7). It is very soluble in hot or cold water (10) and dissolves with the evolution of heat (7).

Gallium hydroxide [$Ga(OH)_3$] is formed by the reaction of bases on solutions of gallium salts or by the action of acids on solutions of gallates (7). For example, when $GaCl_3$ is titrated with a strong base such as NaOH, the precipitation of $Ga(OH)_3$ appears after the additon of 2.8 moles of NaOH and five reaction stages can be distinguished in the range of molar ratios of NaOH:$GaCl_3$ from 0.5-4.0. In the first reaction stage, the addition of one equivalent of NaOH produces a soluble basic salt which dissociates into the ions $Ga(OH)Cl_2 \rightleftarrows Ga(OH)^{2+} + 2\ Cl^-$. When two equivalents of NaOH are added a soluble basic salt is formed which dissociates into the ions $Ga(OH)_2^+$ and Cl^-. In both cases the solutions are transparent with neither $Ga(OH)_3$ nor its salt being formed. With the addition of 2.0-2.8 equivalents of NaOH, a precipitate composed of $Ga(OH)_{2.8}Cl_{0.2}$ is formed and after 2.8-3.0 equivalents of NaOH, $Ga(OH)_3$ precipitates, which can partially exist in colloidal state. With the addition of excess NaOH it is possible to dissolve the $Ga(OH)_3$ and the resulting solution would contain ions, primarily in the form of $[Ga(OH)_4]^-$, but also as $[Ga(OH)_6]^{3-}$, when the NaOH:$GaCl_3$ ratio is greater than four, usually beginning at pH 11.06-11.62 (7).

The pH at which the precipitation of gallium as the basic salt or as the hydroxide starts depends on the $GaCl_3$ concentration, the operating temperature and the nature of the anion of the salt when other gallium salts are used. At a $GaCl_3$ concentration of 0.06-0.18 moles per litre, at

25°C, the precipitation of the basic salt or of $Ga(OH)_3$ has been reported to occur at a pH of 5.01-5.15. As the temperature and gallium concentration rise, the pH at the onset of precipitation decreases [illegible]).

B. Uses of Gallium

The industrial applications of gallium utilize various chemical forms of this element. For example, high purity gallium is used in semiconductors. Gallium arsenide (GaAs) has found use in the production of solar batteries. Gallium-phosphide, due to its high boiling point of 1983°C, is used in rectifiers that are employed for operations at high temperatures. Gallium metal is used for filling quartz thermometers for measuring temperatures between 600°C and 1500°C. Liquid gallium is used in the "cold" soldering of metallic and ceramic materials such as for joining fine wires in heat sensitive instruments.

The uses of gallium in medicine have been confined to the isotopes ^{72}Ga, ^{67}Ga and ^{68}Ga for tumor scanning (7).

C. Biological Studies of Gallium

1. Tissue Distribution of Gallium

Using a chemical method for estimation of gallium in tissues, Dudley (11) observed high kidney and liver concentrations for up to 20 days in the rat following the subcutaneous injection of Ga-lactate, 100 mg/kg. Gallium also entered the bone and was retained there for more than

90 days with only slight loss. After intravenous injection of Ga-lactate in the rat, plasma gallium was initially high but dropped rapidly. Subcutaneous injection produced only a moderate plasma concentration which fell slowly to a constant value after 24 hours. Gallium-72 as the lactate administered intravenously or as the citrate administered subcutaneously was first used to study the bone deposition of gallium in rabbits and dogs using autoradiography (12). Gallium-lactate, at a dose of 8 mg/kg, was found to be selectively deposited in those areas of greatest osteogenic activity, namely the epiphyseal junction, especially in the young animal.

The subcutaneous injection of ^{72}Ga-citrate in rats, dogs and rabbits resulted in only the kidney and bone showing any marked deposition of the gallium (13). Additional studies with ^{72}Ga-citrate administered subcutaneously to rabbits indicated that the epiphyseal junction absorbed four to five times the concentration of gallium as deposited in adjacent bone and also that the callus of healing fracture concentrated gallium to a degree two to three times that found in the bone adjacent to the fracture site (14).

The use of ^{72}Ga in humans was first described by Mulry (15) using patients with proven bone metastasis. Administration of 300-400 μCi of ^{72}Ga as the citrate containing 3.8-5.0 mg of carrier gallium was performed by infusion drip. Geiger counting techniques applied to the skin surface

indicated that the ^{72}Ga was concentrated in the bone lesions by a factor of 20 times compared to levels in adjacent bone tissue. Similar findings were also reported by Lang (16).

The distribution of $^{72}GaCl_3$ (pH 2.0-2.2) was studied in the rat following intravenous administration but no differential uptake was seen in any one tissue although the liver had the highest relative concentration initially which decreased in parallel with the plasma level. Following intravenous administration of ^{72}Ga-citrate to rats, the bone took up the larger fraction of the dose and held it more firmly than the tissues of next highest concentration, namely the liver, spleen and kidney (17).

It was reported by Dudley et al. (18) that gallium chloride was not absorbed from the gastrointestinal tract of rats fed gallium chloride or nitrate at a dose of 1 g of gallium/kg of food over a 13-26 week period. The alkalinity of the intestinal tract was sufficient to convert $GaCl_3$ to the hydroxide or other insoluble complex. Only trace amounts of gallium nitrate were detected chemically in the liver, spleen and kidney. Similar results were obtained when gallium citrate was fed to rats at a dose of 1 g/kg of food (19).

The tissue distribution of ^{67}Ga-citrate after intravenous administration in the rat was first reported by Bruner et al. (20) who found that the quantity of gallium administered as carrier gallium influenced the manner in

which the metal was distributed and excreted. This is shown in Table I. The authors point out that the results observed at the 2.5 and 25.0 mg Ga/kg dose range are similar to those reported previously for ^{72}Ga (17).

Increased ^{72}Ga-citrate tumor uptake following intravenous administration was found in only 8 out of 14 patients known to have definite bone metastases (21). It was therefore concluded that ^{72}Ga did not appear to be too prominsing as a clinical diagnostic tool.

Konikowski et al. (22) investigated the tissue distribution of ^{67}Ga-citrate, lactate, chloride and DTPA complex following intravenous administration in tumor bearing mice. The chloride was adjusted to a pH of 3.0 since earlier work had shown that $GaCl_3$ would begin precipitating as the hydroxide at pH 3.5-4.0. The chloride, citrate and lactate compounds displayed similar tumor uptakes, while tumor concentrations of the DTPA complex were considerably lower. The citrate and lactate showed similar liver uptakes during the time period of study. Liver uptake of the chloride, 30 minutes after injection, was about three times that of the citrate and lactate. The Ga-DTPA complex levels in the liver were very low.

^{68}Ga-EDTA was first proposed for brain scanning by Anger et al. (23). Studies in the rat showed approximately 0.05% of the dose accumulated in the brain one hour after intravenous administration. None of the other organs,

TABLE I

Distribution of ^{67}Gallium-citrate in Rats Five Days After Intravenous Injection[a]

	Carrier Dose in mg Ga/kg					
	0.00	0.0025	0.025	0.25	2.5	25.0
Percent of Dose Excreted						
In urine	0.51	0.49	0.52	0.67	0.81	0.91
In feces	0.49	0.51	0.48	0.33	0.19	0.09
Percent of Dose Per Total Organ						
Liver	5.81	6.52	6.37	5.88	1.83	0.07
Spleen	1.66	1.46	0.85	1.00	0.31	0.13
Kidney	0.92	0.95	0.81	0.85	0.38	0.19
Heart	0.06	0.07	0.06	0.05	-	-
Skeleton	21.57	23.19	23.63	21.58	21.65	15.72
Plasma	0.16	0.24	0.18	0.25	0.15	0.07

[a] As reported by H.D. Bruner *et al.*, (20)

including the liver, appeared to concentrate the material to any extent. No mention was made of the concentration of the ^{68}Ga-EDTA in the kidneys.

2. Gallium Excretion Studies

Following subcutaneous injection of 90-100 mg/kg of stable Ga-lactate into rabbits 90% of the total gallium excreted was in the urine with the remainder being in the feces (24). When ^{72}Ga-citrate was administrated subcutaneously to rabbits an average of 45% of the injected gallium was excreted in the urine within 16 hours with 0.8%-1.3% of the dose in the intestinal contents and 0.3%-1.1% in the feces after 12-18 hours (13).

In a series of patient studies Mulry (15), after administering ^{72}Ga-citrate intravenously, found that the greatest portion of the radioactivity excreted was within the first six hours after injection reaching negligible values after 24 hours.

Munn et al. (25) reported that a dose of 5 mg/kg of stable gallium-citrate administered to rabbits resulted in a greater portion of the gallium being retained following intravenous injection than by subcutaneous injection. Also, the larger the subcutaneous dose the greater was the retention of gallium, being some 20 times greater at the 45 mg/kg level than at the 5 mg/kg level.

Excretion data for ^{68}Ga-EDTA after intravenous administration to rats was presented by Anger (23). These

results were similar to the results observed by Foreman (26) who injected EDTA as the $CaNa_2$-EDTA complex into rats with the result that about 80%-90% of the injected material passed rapidly out of the vascular system into equilibrium with extravascular space. The biological half-time of the remaining material in blood for both man and rats was about one hour. The principle route of elimination was *via* the kidneys and in man over 95% of the dose was excreted within 24 hours.

Edwards *et al.* (27) reported that following a 2.5 mCi dose of ^{67}Ga-citrate in man the whole-body retention was about 65% that of the injected dose after seven days. Urinary excretion was greatest for the first 48 hours with 20%-30% of the total excreted dose being eliminated within that time.

3. Gallium Toxicity Studies

The acutely toxic dose, LD_{50} (10 days), for stable Ga-lactate administered subcutaneously to rabbits was reported to be 480 mg lactate/kg body weight (28). Dudley (18) later reported the LD_{50} (10 days) for stable Ga-lactate in rats and rabbits to be:

In Rats:	intravenous injection	47 mg Ga/kg
	subcutaneous injection	121 mg Ga/kg
In Rabbits:	intravenous injection	43 mg Ga/kg
	subcutaneous injection	97 mg Ga/kg

Perkinson et al. (29) reported that an intravenous dose of stable gallium of 8-10 mg/kg in rats produced an inhibitory effect on the oxygen consumption of the liver lasting 6-12 hours. Stable gallium plus ^{72}Ga (1.7 mCi/kg) produced a greater decrease lasting from 18-24 hours. No histologic damage was seen at either dose.

Bruner et al. (30) showed that if enough ^{72}Ga-citrate were injected intravenously into rats and dogs they displayed opisthotonus and died of acute respiratory paralysis. Administration of calcium prior to the ^{72}Ga-citrate prevented these deaths. The LD_{50} (10 days) of ^{72}Ga-citrate administered intravenously to the rat was found to be greater than 220 mg Ga/kg body weight with the $LD_{10,50,90}$ (15 days) in dogs being 10.5, 18.2 and 41.1 mg Ga/kg respectively. Vomiting, anorexia, debilitation and weight loss were common signs soon after injection. At autopsy the kidneys were enlarged and pale. The tubular destruction ranged from cloudy swelling to severe necrosis with sloughing and blockade. All the lymph nodes were about three times the normal size and form. The immediate cause of death was uremia secondary to acute damage to the renal tubules (30).

The LD_{50} (10 days) for the subcutaneous administration of stable gallium-citrate to a number of animal species as

reported by Dudley *et al.* (19) are:

600 mg/kg (albino mouse)

220-240 mg/kg (50-100 g rat)

100 mg/kg (100 g rat)

45 mg/kg (2.0-2.5 kg rabbits)

10-15 mg/kg (dogs and goats)

The LD_{50} for sodium citrate and sodium lactate in mice following intravenous administration have been found to be 115 mg/kg body weight and 500 mg/kg body weight respectively (22).

In the evaluation of ^{72}Ga-citrate as a therapeutic agent for the treatment of skeletal lesions in man, doses of 10-100 mCi of ^{72}Ga and stable gallium were given by intravenous drip. Toxic manifestations reported were:

(i) profound bone marrow depression characterized by decreased white blood cell, platelet, and red blood cell counts with hypoplasia of the marrow elements probably due to radiation effects

(ii) skin rashes such as folliculitis, extensive maculopapular rash, exfoliative dermatitis and itching due to toxicity of the stable gallium

(iii) gastrointestinal tract symptoms manifested as anorexia, nausea, and vomiting resulting from both radiation and stable gallium effects (31).

^{68}Ga-hydrous ferric-oxide colloid administered intravenously to rabbits and dogs at doses up to 10 mg Fe

per kg body weight or 10 times the proposed human dose have not produced any notable adverse reactions. No significant histological abnormalities were detected in the lungs, adrenals or kidneys (2). Doses of ^{68}Ga-$Fe(OH)_3$ up to several thousand times higher than those expected to be administered to humans produced no visible effects in mice up to 30 days after intravenous injection (32,33).

D. Protein Binding Properties of Gallium

Using a variety of techniques, Hartman and Hayes (34) attempted to demonstrate that the lack of early specific localization in bone at low levels of gallium in the citrate form was due to the binding of gallium by serum components. Using ultrafiltration they found that a membrane capable of retaining solutes with a molecular weight greater than 10,000 retained 80% of the ^{72}Ga-citrate in serum and only 58% of the ^{72}Ga-citrate in normal saline. Using gel filtration it was observed that ^{72}Ga-citrate in serum was eluted in the early fraction where protein with a molecular weight greater than 5,000 normally would be eluted. ^{72}Ga in normal saline was eluted only in the later fractions and then only to a limited extent. With equilibrium dialysis the degree of ^{72}Ga binding with rabbit, human and rat serum was found to be 91.0%, 98.8% and 97.7% respectively.

Gunasekera *et al.* (35) further investigated gallium binding by serum proteins by injecting patients with 2.5 mCi

of ^{67}Ga-citrate intravenously. Blood samples used for their experiments were taken at 3, 24 and 72 hours after injection.

The results of their ultrafiltration study are summarized in Table II. It was concluded from these results that another protein in addition to albumin was responsible for the major binding of gallium. By using electrophoresis and later cross-electroimmunodiffusion techniques with specific antisera, Gunasekera and co-workers demonstrated that the protein responsible for most of the ^{67}Ga-binding was transferrin (35). Gallium, like iron, could be removed from its binding site in transferrin by phosphates (35).

E. Radioisotopes of Gallium Used in Nuclear Medicine

Due to production and/or half-life limitations ^{67}Ga, ^{68}Ga and ^{72}Ga are the only isotopes of gallium that have been investigated for possible medical applications (36).

1. Gallium-72

Gallium-72 was the isotope of gallium initially used for studies in animals and man. Being reactor produced from natural gallium, preparations of ^{72}Ga also contained stable gallium as a contaminant. As previously discussed, the amount of carrier gallium in an administered dose significantly influenced the tissue distribution.

2. Gallium-67

Gallium-67 has a physical half-life of 78 hours and decays by electron capture with the emission of four

TABLE II

Ultrafiltration of Serum

Containing ^{67}Gallium-citrate

Serum Sample	Percent Retained on Membrane
3 hour postinjection serum	85 ± 6.7
24 hour postinjection serum	97 ± 3.2
72 hour postinjection serum	99 ± 1.9
^{67}Ga in normal saline	15 ± 2.2
4% Human Serum Albumin plus ^{67}Ga-citrate	3.5 ± 2.4

main gamma rays; 93 Kev (40%), 184 Kev (24%), 296 Kev (22%) and 388 Kev (7%) (37).

The production of ^{67}Ga by the reaction $^{68}Zn(p,2n)^{67}Ga$ has been described as also producing ^{66}Ga as an impurity through the reaction $^{66}Zn(p,n)^{66}Ga$. This necessitates a four to five day waiting period to allow the ^{66}Ga to decay to about 1% of the ^{67}Ga radioactivity (37). Other methods of production of ^{67}Ga have been reported such as by the reactions $^{70}Ge(\gamma,p2n)^{67}Ga$ (38) and $^{65}Cu(\alpha,2n)^{67}Ga$ (39). In the latter case, stable iron was produced as an impurity. The presence of iron in a ^{67}Ga preparation inhibited the absorption of gallium into tumors (40). A method was proposed for the removal of the iron from the ^{67}Ga by the reduction of Fe(111) to Fe(11) with iodide in concentrated HCl and subsequent percolation through a cation exchanger (40).

a. Clinical Studies with Gallium-67

Clinical investigations with ^{67}Ga were initially started in patients who had proven malignant disorders and known or suspected bone lesions (36). In an effort to obtain favorable bone uptake scanning was begun 24 to 48 hours after the injection rather than by the addition of carrier gallium, as was done for ^{68}Ga-citrate, where scanning was started soon after injection due to the short physical half-life of the radioisotope (41). It was during this preliminary investigation that the localization of

^{67}Ga in a soft tissue tumor was noted in a patient with Hodgkin's disease. Further investigations revealed soft tissue localization in three out of four patients with Hodgkin's disease (42). A pulmonary metastatic lesion was also demonstrated with ^{67}Ga (42). Many investigations have since been undertaken in a variety of soft-tissue tumors in an attempt to explain the mechanisms involved in the tumor uptake of ^{67}Ga. Some of these results are summarized in Table III.

3. Gallium-68

The decay of ^{68}Ge to ^{68}Ga was studied by Crasemann et al. (65). The half-life of the ^{68}Ge was estimated to be 275 ± 20 days. Horen (66) later concluded from his investigations that ^{68}Ge decayed to the ground state of ^{68}Ga by 100% electron capture and that no gamma rays were associated with this decay. The production of ^{68}Ga by the reaction $^{65}Cu(\alpha,n)^{68}Ga$ has been reported in the literature (67,68,69). ^{68}Ga production from the bombardment of zinc targets with 6.3 Mev protons has also been reported (70).

a. Decay characteristics of ^{68}Ga

Mukerje et al. (71) observed the emission of two positrons in the decay of ^{68}Ga with maximum energies of 1.88 ± 0.02 and 0.77 ± 0.02 Mev respectively. They also noted the annihilation radiation (0.511 Mev) and a higher energy gamma component of 1.10 Mev with relative intensities of annihilation:gamma, 17.6:1.0. Various other investigators

TABLE III

^{67}Gallium Localization in Soft Tissue Tumors

Tumor Type	^{67}Ga-citrate Dose	Scan Interval	Comment[a]	Reference
Palpable lymph nodes, Hodgkin's Disease	2.5 mCi	72 hours	1	41
Pulmonary metastasis	2.5 mCi	24 hours	1	41
Experimental tumors in rats and mice			2	43
Malignant lymphoma	2.0 mCi	24 hours	3	27
Reticulum cell sarcoma	2.9 mCi	3 days		
Hodgkin's Disease	2.5 mCi	1-2 days		
Bronchial carcinoma	1.7 mCi	24 hours		
Metastatic carcinoma	3.8 mCi	24 hours		
Ewing's sarcoma	2.0 mCi	16 hours		
Implanted epidermoid carcinoma in rabbits	500 µCi		4	44
Tumor-bearing mice and rats			5,6	45,46

...continued

TABLE III (continued)

Tumor Type	^{67}Ga-citrate Dose	Scan Interval	Comment[a]	Reference
Hodgkin's Disease	1.5-3.0 mCi	72 hours	7	47
Metastatic hepatoma Epithelial cell tumor Uterine carcinoma Hodgkin's granuloma Follicular lymphoblastoma	4.0 mCi	2-6 days	8	48
Cervical carcinoma Endometrial adenocarcinoma Ovarian carcinoma Uterine carcinoma	2-4 mCi	3 hours and 1-9 days	9	49
Myeloblastomas of the breast	35 µCi/kg	48 hours	10	50
Inflammatory lesions	2.5 mCi	48 hours	11	51

...continued

TABLE III (continued)

Tumor Type	^{67}Ga-citrate Dose	Scan Interval	Comment[a]	Reference
Benign, malignant and inflammatory lesions of the lung, mammary gland, stomach, esophagus, colon, pancreas, liver, maxillary sinus, salivery gland, and thyroid gland	1.5-2.0 mCi	48 hours	12	52
Ascites tumor transplanted into mice	0.2 µCi		13	53
Lung and thyroid diseases	2.0 mCi	3 days	14	54
Various malignant conditions	2-3 mCi	2-3 days	15	55
Bronchial carcinoma	2.0 mCi	2-3 days	16	56
Colonic and rectal tumors	variable	24 hours prior to surgery	17	57

...continued

TABLE III (continued)

Tumor Type	^{67}Ga-citrate Dose	Scan Interval	Comment[a]	Reference
Liver cancer	1.5-2.0 mCi	3-4 days	18	58
Liver cancer	3-5 mCi	2-3 days	19	49
Various malignancies	2.5 mCi	2-3 days	20	60
Pulmonary Sarcoidosis	3.0 mCi	48 hours	21	61
Staphyloccocal abscesses in rats	100 µCi/kg		22	62
Rheumatoid arthritis, Paget's Disease, Pulmonary abscesses, fractures, Hepatic abscesses	2.5-3.0 mCi	72-96 hours	23	63
Pneumonitis, osteomyelitis, craniotomy site			23	63
	35 µCi/kg	48-72 hours	24	64

TABLE III (continued)

COMMENTS[a]

(1) Mechanism unknown; could be related to the protein-binding property of gallium.

(2) The uptake of gallium in soft-tissue tumor is associated with viable tumor tissue. Gallium was found in the cytoplasm of neoplastic cells.

(3) The localization of gallium in viable tumor cells suggests an active metabolic process. The decreased blood supply to sites of fibrosis and necrosis may result in the poor uptake in necrotic tissues. Gallium may be bound more tightly to some agent in tumor than it is to circulating protein, or there may be a different agent in the neoplastic cells that binds the gallium in direct competition with the binding agent in normal cells. The tumor may also concentrate the binding agent from the other tissues, causing a decreased uptake of gallium in the liver, spleen and skeleton.

(4) Gallium binds with serum protein, extravasates as a result of increased permeability, enters the tumor cell in an ionic form and binds with the cytoplasm.

(5) The active form of gallium in tumor uptake is ionic. The citrate only prevents the formation of a colloid.

(6) Both gallium citrate and nitrate have similar tumor affinities toward malignant tumor when carrier-free. If carrier gallium is added, both have a weak tumor affinity. ^{68}Ga-EDTA has weak tumor affinity and is excreted rapidly. The chemical form suitable for scanning should be carrier-free and able to be converted into gallium ions in the body.

(7) Gallium seems to be bound to a macromolecular agent located within an intracellular granule, possibly a lysosome-like organelle.

...continued

(8) The gallium-citrate complex dissociates at a low pH. The normal liver uptake may be due to hepatic metabolism of the citrate liberating the free gallium which then forms proteinate complexes within the liver. The pH of the intracellular and interstitial fluid around many tumors is lower than around normal tissues due to preponderance of anaerobic to aerobic glycolysis in tumors resulting in a local lactic acidosis. Gallium uptake in tumors could be due to the increase in the gallium-citrate dissociation which occurs when the pH is lowered.

(9) Gynecologic lesions are less receptive to gallium than other soft-tissue tumors. Accumulation is often seen only in certain portions of the tumor, which may reflect an altered blood supply or a change in the metabolic activity within different portions of the tumor.

(10) Uptake of gallium in the leukemic cells of breast myeloblastoma.

(11) Gallium uptake in tumors and inflammatory lesions may indicate that the uptake of gallium is related to protein-binding.

(12) Positive gallium scans obtained in lung tumor, tuberculosis, pneumonia, lung absecesses and pleurisy which later became negative after treatment. The uptake of gallium in tumors may be due to differences in the permeability of tumor cell membrane from that of the normal cell membrane. Gallium is also found to accumulate in the mucous membranes of the stomach and intestine, thereby making it difficult to detect tumors in these regions.

(13) The high concentrations of gallium were in tissues with high proliferation rates. Gallium was not tumor specific.

(14) Gallium uptake also seen in non-malignant lung diseases, probably in those lesions that are accompanied by a reactive growth of the reticulohistiocytic system. Gallium was not tumor specific.

(15) The accumulation of gallium was not dependent on the histological type of tumor as seen by the uptake of gallium in tumors of the lung, thyroid and stomach.

...continued

(16) From a total of 41 proven cases of bronchial carcinoma, 40 gave a positive gallium scan. Uptake was also seen in non-malignant lung diseases. There was no relationship between the gallium uptake in tumor and its histological cell type.

(17) There appeared to be a correlation between the gallium uptake and degree of tumor differentiation, with poorly differentiated tumors having the highest uptake. Gallium uptake in a colonic tumor was at the edge of the tumor, while in the ulcerated center, the uptake was normal, possibly indicating that uptake is related to cellular proliferation within the tumor.

(18) The large uniform deposition of gallium in the normal liver may be due to hepatic metabolism of the citrate liberating the gallium to form a proteinate complex. The visualization of liver cancer with gallium is due to vascular perfusion of the tumor.

(19) A focal liver abnormality that took up more gallium than the surrounding normal liver was more likely due to cancer or abscess than to a benign condition such as cirrhosis.

(20) The relative amount of gallium within the tumor and within the normal tissues seems to depend on the volume of tumor tissue.

(21) It was possible that the gallium activity as seen in the lung in Sarcoidosis was due to the incorporation of gallium into the phagocytic histiocytes that are present in the tubercles of Sarcoidosis which are packed with lysosomes when the disease is active.

(22) As an abscess ages its pH decreases, which could promote the uptake of gallium in the abscess. Also, the lymphatic effluent from the abscess area has been shown to decrease after the initial infection which could permit further retention of the gallium-proteinate complexes.

(23) Awareness of the non-specificity of gallium is important since a localized increase in radioactivity may be wrongly interpreted as a sign of malignancy in the patient who is really free of any neoplastic disease.

(24) Normal ^{67}Ga radioactivity is concentrated in the axial skeleton, liver, spleen and large joints. Uptake is often seen in the salivary, lacrimal and mammary glands as well as in the region of the nasopharynx. Under certain physiological conditions, intense localization may occur within the breast, bowel and long-bones. The lymph nodes are normally not visualized on ^{67}Ga scans and their appearance is an indication of disease.

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[a] Observations as reported by the various investigators

have studied the decay of ^{68}Ga and some of their results are summarized in Table IV and in Appendix 1.

^{68}Ga decays by positron emission (88%) and electron capture (12%) (2). Positron decay is one of two ways in which an unstable atom can convert a nuclear proton to a neutron, $p^+ \rightarrow n + \beta^+$, (72,73). The second way that an unstable atom can convert a proton to a neutron is by the electron capture process. The change within the nucleus is the same whether positron emission or electron capture is the mode of decay and for this reason the two processes compete with each other. Positron active nuclides release the positron with specific energies up to a few Mev. The range in tissue of the positron is similar to that of ordinary electrons or beta particles of the same energy. The positrons travel at most a few millimeters from their site of origin before undergoing annihilation, which occurs at the end of their range when their kinetic energy is reduced almost to zero. At that time, the positron and an electron combine as a result of electrostatic attraction and annihilate each other. The mass of the two particles disappears, being changed into a form of electromagnetic radiation called annihilation radiation. The result is the creation of two photons travelling in opposite directions and each having an energy equivalent to the mass of a single electron, 511 Kev. Photons of this energy suffer no significant attenuation in traversing body structures. A pair of detectors set up in

TABLE IV

The Decay of Gallium-68[a]

Positrons	Gamma Rays	Comment	Reference
B_1=1.88±0.02 Mev[b] B_2=0.77±0.02 Mev[c]	511 Kev annihilation and gamma of 1.10 Mev		71
B_1=1.94±0.05 Mev B_2=0.92 Mev	Gamma of 1.02 Mev	Electron capture-to-positron ratios: B_1=0.1 and B_2=1.1	65
	511 Kev annihilation and gammas of 1.07, 0.81, 1.24 and 1.88 Mev	Total positron branching in the decay of ^{68}Ga found to be 89%	66
		Electron capture-to-positron ratios: B_1=0.10±0.02 and B_2=1.28±0.12	74

... continued

TABLE IV (continued)

Positrons	Gamma Rays	Comment	Reference
	Gammas of 578±1.0 Kev, (1.1%); 805±0.6 Kev, (2.8%); 1077±0.2 Kev, (100%); 1261±0.3 Kev, (2.9%); 1746±1.0 Kev, (0.28%); 1883±0.4 Kev, (4.1%); and 2338±2.0 Kev, (0.4%)	Gamma rays determined with a 15 cc Ge(Li) detector in conjunction with a 4096 channel analyzer and with a Ge(Li)-NaI(Tl) coincidence arrangement	75,76
		The electron capture of ^{68}Ge (100%) to the ground state of ^{68}Ga establishes a 68.3 minute half-life of ^{68}Ga in secular equilibrium	76
B_1=1.899 Mev B_2=0.75-0.93 Mev			77

...continued

TABLE IV (continued)

Positrons	Gamma Rays	Comment	Reference
B_1=1.898 Mev (86.67%) B_2=0.820 Mev (1.50%)	Gammas of 0.80, 1.078, 1.24, 1.87, 2.32 Mev	A total of four electron capture processes	78
B_1 and B_2 account for 88% of the decay emissions of ^{68}Ga	Less than 4% decay due to other gammas	12% decay due to electron capture processes	2

[a] As determined by various investigators

[b] B_1 = decay to the ground state

[c] B_2 = decay to the first excited level

a coincident circuit can locate and identify the site of positron emission through coincidence registration of the two incident photons. Due to this coincidence type of detection, collimation requirements can be reduced by a large factor, even to zero in some cases as is the case with the positron camera. Since no collimators are required, low amounts of radioactivities can be administered to the patient and detected by the positron camera. This instrument has a high sensitivity and yields good resolution for structures deep within the patient (72,73).

b. The Gallium-68 Generator

The decay of the 275 day half-life ^{68}Ge has been utilized for the production of a ^{68}Ga generator system. Most positron emitting radioisotopes are produced by irradiation in a cyclotron making them of limited availability to those not near such a facility. Also the high costs of the cyclotron produced positron emitters prohibits their use in many institutions. Gallium-68 is one of the few useful positron emitting isotopes that can be obtained from a generator system (4).

Gleason (4) was the first to report on the production of such a generator system using ^{68}Ge as obtained from the reaction $^{69}Ga(p,2n)^{68}Ge \rightarrow {}^{68}Ga$. The ^{68}Ge was recovered from the irradiated material in a radiochemically pure state. A solvent extraction procedure was used to separate the ^{68}Ga from the ^{68}Ge using acetylacetone which extracted the

carrier-free ^{68}Ga from a slightly acidic solution of ^{68}Ge. A faster method of separating the ^{68}Ga from the ^{68}Ge was described by Green et al. (5) using chromatographic alumina as an absorbent for the ^{68}Ge. The generator consisted basically of an alumina column onto which the ^{68}Ge had been adsorbed. The ^{68}Ga was eluted from the column with 0.005M EDTA, pH 7.0 in the chemical form of ^{68}Ga-EDTA complex. Contamination by ^{68}Ge in the eluate was less than 3×10^{-4}% of the ^{68}Ga radioactivity at time of elution (5). Yano (6) adapted the above generator system so that the ^{68}Ga-EDTA eluate could be collected in a sterile and pyrogen free form suitable for immediate use.

Gallium-68, being generator produced, offers the following advantages in Nuclear Medicine procedures (2):

(i) the 68.3 minute half-life of ^{68}Ga reduces the radiation exposure to the patient

(ii) the short half-life also permits the rapid build-up to equilibrium amounts in the generator from which the ^{68}Ga can be eluted every three to four hours

(iii) the high yield of positron emission (88%) and the resulting 511 Kev annihilation gammas permit coincidence detection with the positron camera eliminating the need for collimation; this reduces the amount of radioactivity needed for a particular study with the advantage of reducing the radiation exposure to the patient

(iv) the long half-life of the ^{68}Ge (275 days) extends the useful life of the generator over a period of many months, thereby reducing the cost of the ^{68}Ga; the initial cost being prorated over the useful life of the generator

(v) the ^{68}Ga in the form of ^{68}Ga-EDTA can be used as such for brain scanning or the complex can be dissociated by various methods in order that the free ^{68}Ga may be used in the preparation of other radiopharmaceuticals, some of which are listed in Table V

(vi) positron emitters offer possibilities of better resolution with coincidence detection systems than is obtainable with single detector systems (79).

Any generator system must yield a daughter nuclide of high purity with respect to both radioactive and stable contaminants. Such a high level of purity must be maintained throughout the useful life of the generator (80). Radionuclidic purity is the most important consideration of a generator system since with the passage of time, a minor long-lived impurity may become the predominant radionuclide present, adding to the radiation dose of the patient. Chemical impurities present no real hazard to the patient unless they are present in sufficient quantity to be chemically toxic (89).

Purity tests have been conducted on the ^{68}Ga

TABLE V

GALLIUM-68 RADIOPHARMACEUTICALS

Organ	Chemical Form	Dose	Comment[a]	Reference
Brain	^{68}Ga-EDTA	250 µCi	Useful pictures were obtained 10 minutes after the injection of the isotope. The labelled EDTA may be of use in brain tumor localization in situations where increased permeability of the blood-brain barrier exists.	23
Brain	^{68}Ga-EDTA	700-750 µCi	^{68}Ga-EDTA reported to be as successful as ^{203}Hg-Neohydrin for detecting brain tumors but was not as tumor specific as ^{203}Hg.	81
Brain	^{68}Ga-EDTA	400-500 µCi	Pictures were taken 10 minutes after the injection with exposure times ranging from 4 to 10 minutes. Of 50 cases with abnormal uptakes, 41 were confirmed abnormal by surgery or autopsy.	82

...continued

TABLE V (continued)

Organ	Chemical Form	Dose	Comment[a]	Reference
Bone	^{68}Ga-citrate	300 µCi	Studies in the rat using ^{68}Ga-citrate containing 5 mg gallium/kg as carrier resulted in good skeletal definition compared to the same compound without the added carrier which resulted in a whole-body distribution without good skeletal localization. Both scans were obtained one hour after the injection. Requisite for the use of ^{68}Ga in clinical bone scanning would be rapid localization in bone coupled with a rapid clearance from other tissues.	36,41
Bone	^{68}Ga-citrate	-	Positive findings on the scans corresponded to known or subsequently demonstrated skeletal lesions. Several lesions were detected on the scans before they were demonstrated on x-rays. Fracture, myelofibrosis, and inflammatory or degenerative arthritis also gave increased uptake of the isotope.	83

...continued

TABLE V (continued)

Organ	Chemical Form	Dose	Comment[a]	Reference
Bone	^{68}Ga-citrate	-	The addition of carrier gallium from 2-4 mg/kg body weight resulted in enhanced skeletal uptake.	84
Bone Marrow	^{68}Ga-hydrous ferric oxide colloid	-	Localized rapidly in the liver, spleen and bone marrow of test animals. Bone marrow:bone ratio = 100; bone marrow: blood ratio = 200; bone marrow:liver ratio = 1.5. The disappearance half-time of the colloid from the blood in rabbits was found to be 9 minutes, which was rapid enough to begin scanning before too great a portion of the radioactivity had decayed.	85,86
Bone Marrow	^{68}Ga-hydrous ferric oxide colloid	-	Compares favorably with ^{99m}Tc-sulfur colloid for bone marrow scanning. The blood clearance half-times in patients for ^{68}Ga = 2 minutes; for ^{99m}Tc = 7 minutes. After 30 minutes 1% of the ^{68}Ga dose remained in the blood, compared to 10% of the ^{99m}Tc dose.	2

...continued

TABLE V (continued)

Organ	Chemical Form	Dose	Comment[a]	Reference
Lung	^{68}Ga-$Fe(OH)_3$ particles	100 µCi	Particles formed were between 10 and 30 microns in diameter. Clearance half-time from the lungs in mice (using ^{67}Ga) was found to be 30 hours, at which time 50% of the dose was then in the liver. Perfusion tomograms in humans might be of value in determining the size and location of perfusion abnormalities in the lung.	32,33
Kidney	^{68}Ga-poly-metaphosphate Mg-polymeta-phosphate	-	Tissue distribution in rats one hour after intravenous administration expressed as percent of injected dose was: kidney = 40.8; liver = 3.1; blood = 1.9/ml.	87
Liver	^{68}Ga-chromic phosphate	300 µCi	The tissue distribution in rats one hour after intravenous administration expressed as percent of injected dose was: liver = 90.55; kidney = 2.25; spleen = 3.92; lung = 0.54; blood = 0.86/ml; bone = 0.34/gm (femur).	88

[a] Results and observations as reported by the various investigators

generator developed by Greene and Tucker (5) in an attempt to determine the presence and extent of ^{68}Ge contamination in the eluate and also the presence of other radionuclide impurities (89). The results of these investigations indicated no radionuclidic impurity other than ^{68}Ge which was found to be present to the extent of 0.043% (after the first elution) to 0.004% (after the eighth elution) of the total ^{68}Ga radioactivity initially present. The amount of ^{68}Ge contamination decreased after each subsequent elution.

c. Separation of ^{68}Ga from the ^{68}Ga-EDTA Complex

For the preparation of ^{68}Ga radiopharmaceuticals other than ^{68}Ga-EDTA it is necessary to separate the ^{68}Ga from the ^{68}Ga-EDTA complex. Various procedures for this separation have been reported in the literature. One such method described by Hayes (41) involved the extraction of the ^{68}Ga-EDTA eluate with isopropyl ether after addition of 7.5N HCl to the ^{68}Ga-EDTA eluate. The resulting $^{68}GaCl_3$ was then extracted with distilled water.

Yano (6) used a procedure that required addition of carrier $GaCl_3$ in HCl to the generator eluate. Saturated ammonium acetate was then added followed by the addition of concentrated NH_4OH which precipitated the $Ga(OH)_3$. The mixture was heated, centrifuged and the $Ga(OH)_3$ dissolved in 20% NaOH. Using 10 mg $GaCl_3$ carrier, about 60% of the ^{68}Ga from the EDTA complex was recovered. With 20 mg $GaCl_3$,

about 70% recovery was obtained. A third method described by Weber *et al.* (90) consisted of evaporating the ^{68}Ga-EDTA eluate to dryness in a platinum crucible under an infrared lamp, ashing at about 400°C for 20 minutes and dissolving the ash in 2% citric acid.

A less time-consuming method using a process of ion-exchange was described (91). The ^{68}Ga-EDTA eluate was added to an equal volume of 6N HCl causing dissociation of the complex. The mixture was then passed through an anion exchange resin which retained the free ^{68}Ga while allowing the EDTA to pass through. The ^{68}Ga was then removed from the resin by the addition of 0.1N HCl. Recovery of the ^{68}Ga using a Bio-Rad AG 1-X2 anion exchange resin was 98% - 99%, while for AG 1-X4 and AG 1-X8 resins, slightly lower recoveries were obtained. The resin AG 1-X10 yielded a recovery of only 85%.

4. Radiation Dosimetry for the Radioactive Gallium Isotopes

The radiation dose from ^{67}Ga depends on its organ distribution as well as on its effective half-life in the organs of interest (55). By using the assumption that "There is the same relative uptake per gram of tissue in proportion to total body weight, in corresponding organs in rat and man", Popham *et al.* (92) attempted to estimate the radiation dose in man by extrapolation from the retention pattern of ^{67}Ga in rats. From whole-body retention measurements in rats they showed that an injection of seven μCi of

^{67}Ga-citrate was retained to the extent of 40% in the body for two to four weeks after administration. They predicted that the administration of 2.5 mCi in man, assuming no biological clearance after two weeks with only physical decay of the isotope, would result in a radiation dose of two rads to each of the liver, spleen, kidney, testis and skeleton with a whole-body dose of about 0.5 rads. Lavender et al. (51) reported that following an intravenous injection of 2.5 mCi of ^{67}Ga-citrate in man, the total-body absorbed radiation dose was 0.85 rads with the critical organs, bone and kidney each receiving about 4.5 rads. The whole-body effective half-time in the rat following an intravenous injection of eight μCi of ^{67}Ga was estimated to be 73.5 days with a biological half-life of 53.1 days (55). Gallium retention in two tumor-free patients was measured and biological half-times of 14 and 22 days, respectively, were observed (55). Edwards and Hayes (27) reported that the administration of 2.5 mCi of ^{67}Ga-citrate to patients, resulted in an absorbed radiation dose to the whole-body of 0.3 rads/mCi, assuming no excretion and uniform distribution. The radiation dose to the bone was estimated as 2.0 rads/mCi assuming 100% deposition in bone with no excretion. The whole-body retention was about 65% of the injected radioactivity after seven days. An injection of 2.5 mCi ^{67}Ga-citrate in humans gave a total-body radiation dose of less than one

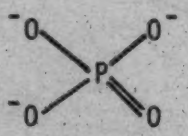
rad, with the kidneys and bones each receiving four rads (60).

Brain scanning with 250 μCi of ^{68}Ga-EDTA in humans has been reported to give a whole-body radiation dose of less than seven mrads and a renal dose of less than 50 mrads (5). Gottschalk *et al.* (81) reported that brain scanning with 700-750 μCi of ^{68}Ga-EDTA gave a whole-body radiation dose in humans of less than 30 mrads with a renal dose of less than 150 mrads. When ^{68}Ga-hydrous ferric oxide colloid was used for bone marrow scanning, the radiation dose to the bone marrow, liver and spleen in humans, based on animal studies, was 1.6, 0.75 and 1.5 rads/mCi respectively (85). Anghileri (87) studied the distribution of ^{68}Ga-polymetaphosphate-Mg-polymetaphosphate in rats and estimated the renal dose to be about ten rads per mCi compared to ^{197}Hg-chlormerodrin which gave a renal radiation dose of 34.2 rads/mCi. Approximately 10% of the administered radiomercury was retained in the kidneys with a biological half-life of 28 days. ^{99m}Tc-iron complex gave a renal dose of 500 mrads per mCi with 20% of the administered dose being retained in the kidneys after 24 hours (87).

II. Polyphosphates

A. Chemistry

The phosphates are those compounds of phosphorus in which each atom of phosphorus is surrounded by four oxygen atoms arranged at corners of a tetrahedron. Polymers of the phosphates such as the polyphosphates can be formed by sharing oxygen atoms between tetrahedra to form the so-called condensed phosphates (93,94).



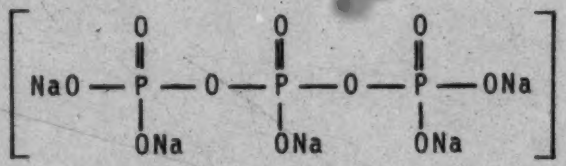
$(PO_4)^{3-}$

Phosphate tetrahedron (orthophosphate)

The condensed phosphates include the chain, ring and branched polymers formed by the repeated condensation of tetrahedral phosphate groups. Included in this class of phosphates are the linear polyphosphates $(P_nO_{3n+1})^{(n+2)}$, such as the pyrophosphates, (n = 2), the oligophosphates (n = 5 to 10) and the long-chain phosphates which include maddrell, kurrel and Grahams salts with average chain lengths from about 200-10,000 or more PO_4 units, depending upon the method of chemical preparation (93,94).

The tripolyphosphate, of which the sodium salt is the best characterized, is manufactured from a mixture of one mole of monosodium orthophosphate and two moles of disodium orthophosphate intimately mixed and calcined at a temperature between 300°C and 900°C. Conversion to

the sodium tripolyphosphate is rapid at these elevated temperatures but the rate of conversion does not affect the properties of the product (95).



Sodium tripolyphosphate ($Na_5P_3O_{10}$) (95)

Sodium tripolyphosphate exists in two anhydrous forms (phase I and phase II) and as the hexahydrate ($Na_5P_3O_{10} \cdot 6H_2O$) (95,96). In phase II, all of the sodium ions are octahedrally coordinated by oxygen. In phase I, some of the sodium ions are surrounded by only four oxygen atoms. Phase I is the more rapidly hydrating form and when added to water lumps or cements together due to its extremely high solubility. Phase II dissolves less readily in water.

As the chain lengths of the polyphosphates increase, it becomes increasingly difficult to recrystallize the phosphates from aqueous solutions when treated with large volumes of alcohol or acetone (96). For example:

(a) Na_2HPO_4 + alcohol → precipitates as a crystalline substance

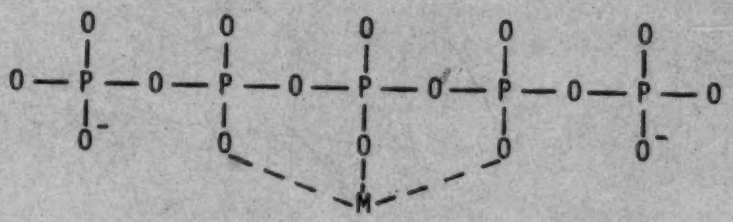
(b) $Na_4P_2O_7$ + alcohol → precipitates as a gummy crystal which eventually solidifies into a hard mass

(c) $Na_5P_3O_{10}$ + alcohol → precipitates as an oil which is slowly transformed into crystals

(d) $Na_6P_4O_{13}$ + alcohol → precipitates as an oil which does not crystallize on standing

The hydrolysis of the chain and ring phosphates in aqueous solution is very slow, the half-life of these P-O-P linkages with respect to hydrolysis at neutral pH and room temperature is of the order of magnitude of years but at very high temperatures the polyphosphates degrade completely to orthophosphate (96). The hydrolysis of the polyphosphates is catalysed by hydrogen ions (97).

The phosphates are highly charged anions and tend to associate strongly with cations in all but the most dilute solutions. This complexing ability involves both ionic and covalent attractions for the final bond formation. The polyphosphates are typical polyelectrolytes (96,98). The alkaline earth metal ions form phosphate complexes more readily than the alkali metal ions and the orthophosphate complexes of the alkali and alkaline earth metals are weaker than the equivalent complexes of the chain or ring phosphates. Transition group metals form very strong complexes and the degree of complexing increases with the charge on the metal ion being complexed. The geometry of the chain phosphates is such that an oxygen atom from each of three neighboring phosphate groups can bind with a metal to form the complex (96,98).



Metal Complex of the Chain Phosphate (98)

When a polyphosphate solution is gradually added to a solution containing polyvalent metal ions, a precipitate is first formed which dissolves upon the addition of more polyphosphate. All polyphosphates form insoluble salts with polyvalent metal ions and these salts can be dissolved by the formation of soluble complexes in the presence of excess polyphosphate anions (95).

B. Uses of the Phosphates

1. Industrial Uses

The phosphates have been used in phosphate fertilizers, animal feeds, as water softening agents, synthetic detergent builders, and in various other applications. A detailed discussion on the uses of the phosphates is presented by Van Wazer (99).

The ability of the phosphates to sequester calcium and magnesium in hard water is due to their interaction with metal ions to form soluble complexes (96).

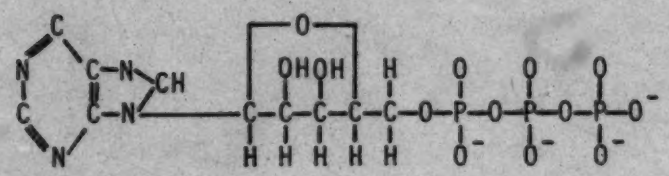
In the food industry, orthophosphate is used to complex iron in soft drinks, jams and jellies thus preventing the dulling of the colors of the naturally occuring

vegetable dyes (98).

Due to their high negative charge, the phosphates are strongly adsorbed on surfaces and can greatly affect suspensions of colloidal particles. The chain phosphates are well known for their peptizing, defloculating and dispersing properties (98).

2. Biological Functions

The tripolyphosphates of major biological interest are adenosine triphosphate and the related 5'-ribonucleotides.



Adenosine Triphosphate (96)

Included in this class of tripolyphosphates are the inosine, cytidine, uridine and guanosine triphosphates (96).

Phosphorus is universally found in protoplasm and is essential for growth and reproductive processes as well as for maintaining the health of all plants and animals. Some of the chemical effects on living systems of the phosphates include: the entrance of polyphosphate monoesters into chemical reactions so as to cause them to proceed, pH buffering and formation of soluble complexes

with cations and precipitation of orthophosphate ions with calcium to give the highly insoluble hydroxyapatite which forms the basis of bone (99).

3. Phosphates in Medicine

Normal cells of different tissue structures have varied metabolic and turnover rates and diverse phosphorus requirements (100). The retention of ^{32}P has been shown to decrease in the following order: bone, liver, intestine, heart, kidney, lung, muscle, skin, and brain (100).

Marshak (101) has shown that the nuclei of malignant cells concentrated ^{32}P to a greater extent than the nuclei of normal cells. The same effect was also noted in rapidly growing cells. Phosphorus-32 in the form of Na_2HPO_4 has been used for the therapy of osseous metastases arising from carcinoma of the breast. Regeneration of bone and apparent arrest of further bone involvement were noted on X-ray examination one year after the initial therapy, indicative of a palliative effect (100). Maxfield et al. (102) advocated the use of testosterone immediately before and during ^{32}P treatment of bone metastases on the premice that the testosterone would enhance the uptake of the radioactive phosphorus into the area of the bone lesion thereby providing a higher radiation dose at the site of metastases. Using autoradiography, Naplan et al. (103) examined sections of human bone containing lesions that had recently been treated with ^{32}P. They found the ^{32}P

radioactivity to be confined to the periphery of the bone lesion in areas of regenerating bone adjacent to the lesion.

The localization of ^{32}P-labelled trimetaphosphate or polymetaphosphate was investigated in mice and rabbits by Fels *et al.* (104). These condensed phosphates were poorly absorbed from the oral route. Following intravenous and intraperitoneal injection, these compounds localized in bone, particularly in actively growing areas, and were reported to be superior to the orthophosphate (NaH_2PO_4) as ^{32}P donors to bone (104). ^{32}P-polymetaphosphate was further investigated for possible use in the therapy of bone tumors in humans (105). Seven of eight patients given a total dose of 16-20 mCi of the ^{32}P-polymetaphosphate over a five week period showed palliation of pain and clinical improvement. After administration of ^{32}P-polymetaphosphate a marked and persistent deposition was noted in metastatic bone tumors compared with normal bone. The greatest activity was seen in bone spicules rather than in tumor nodules. Its preferential deposition in growing bone lesions was thought to be the result of hydrolysis at the site of the bone lesion due to the high local concentration of alkaline phosphatase at the lesion site (105).

The *in vitro* effects of the acid and alkaline phosphatases on polyphosphates were studied by Anghileri

(106). The acid phosphatase produced the greatest hydrolytic effect on the polyphosphates. Also, the formation of the intermediate lower polyphosphates resulting from hydrolysis was less for the cross-linked polyphosphates compared to the straight-chain polyphosphates. The *in vitro* hydrolysis of polyphosphate by tissues from tumor-bearing animals indicated that the hydrolysis occurred at the terminal phosphate groups (107). Anghileri (108) also studied the metabolic fate of the straight-chain and cross-linked polyphosphates in mice and rats. When administered by the oral route the polyphosphates were completely hydrolyzed in the intestine and absorbed only as the monophosphate. The straight-chain polyphosphate was hydrolyzed by the bone tissue to orthophosphate more rapidly and to a greater extent than the cross-linked polyphosphate. The uptake by bone of the polyphosphates was thought to be due to a preliminary physiochemical adsorption to the bone followed by enzymatic hydrolysis of the polyphosphate with the consequent readsorption or binding of the hydrolytic products (108).

The uptake of polyphosphate and orthophosphate in normal bone and in bone autoclaved for 20 minutes, to destroy any enzymatic activity, was studied by Anghileri (109). The incorporation of the polyphosphate and the orthophosphate, was higher in the autoclaved bone than in the normal bone, indicating that uptake was independent

of biological or enzymatic mechanisms. The higher uptake in the autoclaved bone was attributed to an increase in the active surface of the mineral tissue resulting from the hydrolysis of collagen. The binding of the polyphosphate and orthophosphate to insoluble calcium compounds similar to the bone mineral tissue constituents was also investigated and interpretation of the results indicated that physiochemical processes were responsible for the retention, independent of enzymatic hydrolysis (109).

a. Tissue Distribution Studies

Table VI illustrates the results of two studies performed on the tissue distribution of ^{32}P-labelled linear and cross-linked polyphosphates and ^{32}P-labelled orthophosphate (110, 111).

Eight days after the injection, the uptake of the linear polyphosphate in the bone was essentially the same as after 24 hours. The uptake of the cross-linked polyphosphate in bone was about half that of the linear polyphosphate, presumably as a result of the greater excretion of the cross-linked form due to its lower susceptibility to the action of the phosphatases (110). Even though the uptake of the orthophosphate was about twice that of the linear polyphosphate in bone after intraperitoneal injection, it was also higher in many soft tissues. Therefore, for therapeutic purposes, ^{32}P-polyphosphates would give a reduced total body radiation dose compared to

TABLE VI

Twenty-four Hour Phosphate Distribution in Mice[a]

Tissue[b]	Intraperitoneal Injection		
	Linear Polyphosphate	Cross-linked Polyphosphate	Orthophosphate
Bone[c]	8.89 ± 5.08	9.30 ± 4.15	19.40 ± 2.67
Blood	0.46 ± 0.26	0.28 ± 0.07	0.58 ± 0.07
Liver	1.95 ± 0.51	1.17 ± 0.41	2.68 ± 0.41
Spleen	2.38 ± 1.82	1.97 ± 0.47	6.02 ± 1.01
Pancreas	1.06 ± 0.35	1.17 ± 0.45	2.96 ± 0.95
Kidney	3.06 ± 1.89	1.28 ± 0.45	3.66 ± 0.95
Lung	1.11 ± 0.71	0.73 ± 0.17	1.86 ± 0.78
Muscle	0.91 ± 0.43	0.78 ± 0.20	2.78 ± 1.44
Brain	0.18 ± 0.06	0.18 ± 0.05	0.31 ± 0.03
G.I.T.	2.05 ± 1.04	1.06 ± 0.31	1.63 ± 0.66

...continued

TABLE VI (continued)

Twenty-four Hour Phosphate Distribution in Mice[a]

Tissue[b]	Intravenous Injection		
	Linear Polyphosphate	Cross-linked Polyphosphate	Orthophosphate
Bone[c]	9.82 ± 1.71	9.98 ± 1.71	8.46 ± 1.53
Blood	0.20 ± 0.05	0.16 ± 0.05	0.61 ± 0.10
Liver	1.01 ± 0.36	0.86 ± 0.22	1.48 ± 0.19
Spleen	1.21 ± 0.42	1.06 ± 0.32	2.79 ± 0.47
Pancreas	0.85 ± 0.12	0.91 ± 0.18	1.66 ± 0.39
Kidney	1.10 ± 0.17	0.78 ± 0.21	1.66 ± 0.21
Lung	1.71 ± 1.36	1.21 ± 0.62	1.01 ± 0.24
Muscle	1.66 ± 1.33	1.01 ± 0.62	1.00 ± 0.19
Brain	0.13 ± 0.03	0.12 ± 0.04	0.28 ± 0.03
G.I.T.	0.95 ± 0.28	0.62 ± 0.21	1.45 ± 0.19

[a] From L.J. Anghileri (110) and L.J. Anghileri (111)

[b] Percentage of injected dose per gram of tissue

[c] Femur

orthophosphate due to the lower uptake of the polyphosphate in the blood and in other organs (110,111).

The tissue distribution of the linear form of ^{51}Cr-polyphosphate in mice after intravenous administration was studied and the uptake pattern was found to be: bone > liver > kidney > lungs > pancreas > intestine > muscle > blood > brain (112). The bone uptake was attributed to the complexed chromium atom and a phosphate group of the polyphosphate reacting with the OH^- groups of hydroxyapatite, followed by hydrolysis producing either shorter chains of ^{51}Cr- condensed phosphates or insoluble Cr^{51}-PO_4. These hydrolysis products were either excreted or recombined with the bone tissue (112).

b. Phosphate Toxicity

Reduction in serum calcium levels, particularly in hypercalcemia has been demonstrated following phosphate administration (113). The mechanism involved in the calcium lowering effect was reported to be due to $CaHPO_4$ precipitation. The urinary excretion of calcium did not increase following phosphate administration. Such precipitation of $CaHPO_4$ was reported to be objectionable and potentially hazardous causing damage to soft tissues by calcification (114).

Eisenbert (115) suggested that the administration of phosphate in man did not prevent bone reabsorption, but that the calcium was removed from the circulation and

sequestered into a metabolic pool from which the calcium phosphates were ingested by macrophages and then slowly released. It was postulated that the administration of large doses of phosphate overwhelmed this protective mechanism causing the precipitation of $CaHPO_4$ in soft tissue resulting in soft tissue calcification. Stamp (116) reported that phosphate administration resulted in a decreased plasma calcium in all subjects, normal or hypercalcemic. Prominent calcified subcutaneous masses have appeared at injection sites following the intravenous administration of a 2% phosphate solution (113).

III. Radiopharmaceuticals for Bone Scintigraphy

A. Applications of Bone Scanning

From a recent review of the literature, the bone scan's greatest usefulness was stated to be for the diagnosis of the radiographic or x-ray occult tumor and in outlining the extent of tumor (117). Other potential uses of the bone scan are: the evaluation of the asymptomatic patient with minimal lesion as shown on x-ray films; in differentiating traumatic from pathologic fractures; in evaluating the response to radio- and chemotherapy; and in the staging of tumors (117). Bone scanning is based on a disturbance of the bone mineral metabolism and indicates the dynamic state of the bone. X-ray films show the net changes, both osteolytic and reparative that have

occured (117). The process of tumor growth with destruction of bone and the process of repair in response to the tumor coexist in varying proportions and are visualized on x-ray film as areas of radiolucency and radiodensity respectively (118). X-ray manifestations often are visible only in the later stage of bone involvement and often may not be demonstrable despite localized bone pain resulting from metastases (118).

Metastases involving bone are very common and are reported to be exceeded in frequency only by metastasis to lymph nodes, lungs and liver (118). The frequency of bone involvement from some of the malignant tumors such as those of the lung, breast, prostate, intestine, thyroid and kidney which metastasize to bone was approximately 50%-75% at the time of death prior to 1950. Due to advances in management and prolongation of life since then, the frequency of bone involvement now at death could be as high as 85% (118). Bone tissue usually reacts to the presence of metastatic tumor by forming new bone and bone mineral tracers are incorporated in the skeleton wherever bone tissue is being formed as in the process of growth and remodeling or as a result of trauma and disease (119). It has been shown, for example, with strontium isotopes, that any disorder which actively produces new bone will also give rise to a positive strontium scan (118). Examples of such disorders include: osteomyelitis, fracture,

Paget's disease, eosinophilic granuloma, chondrosarcoma, giant cell tumor, fibrous dysplasia, osseous metaplasia, osteoarthritis and rheumatoid arthritis (120).

The interpretation of the scans of the skeleton, in many instances is a subjective process, since the radionuclides that concentrate at sites of new bone growth also localize to some extent in normal bone (121). Also, radioactivity in the kidneys, bladder and intestinal tract, resulting from the excretion of the radionuclides, may obscure areas of possible bone metastases (121).

B. Radiopharmaceuticals Used for Bone Scanning

A number of radioisotopes and radiopharmaceuticals are currently available for bone scanning. Generally, each nuclear medicine laboratory chooses its bone scanning technique on the basis of; the types and numbers of patients, equipment available, the availability and costs of the radioisotopes, as well as the availability and number of qualified staff (121).

1. Calcium-47

Calcium-47 has a half-life of 4.7 days and emits high energy gamma rays of 1.31 Mev. Lead shields and collimators in most hospitals are not adequate for scanning at this high gamma energy and the isotope is rarely used for bone scanning (118).

2. Strontium-85

Strontium-85 has a half-life of 64 days and decays by the emission of a single gamma ray of 0.513 Mev. The pattern of distribution in the human bone is similar to that of calcium (118). Strontium gains access to bone by exchanging with stable strontium and by substitution for calcium atoms on the surface of the hydroxyapatite crystal lattice of newly forming bone. Less than 1% of the body's calcium stores participate in this process. The rate of strontium uptake by normal bone is rapid and half of the final amount taken up accumulates in 15 minutes (118). Poor results were obtained with the Anger camera due to low detection efficiency for the 513 Kev photons (118). From 20%-30% of the administered dose of ^{85}Sr is excreted into the urine within 10 days, with half of this amount being excreted within the first 48 hours. In order to obtain high bone-to-background ratios, scanning is usually begun 48 hours after administration. About 17% of the injected dose may be excreted in the feces necessitating thorough bowel cleansing prior to scanning. Doses are limited by the Atomic Energy Commission (United States) for the clinical investigation of those patients with diagnosed cancer (118). Administration of 50-100 μCi as the nitrate or chloride, results in a radiation dose to the whole-body of 350-700 mRads and to the bone of 1500-4500 mRads (121).

The primary deterrent to the widespread use of ^{85}Sr is the time required for a scanning procedure, for example, a scan of the pelvis requires 30-45 minutes to complete (120). Due to the long physical half-life of ^{85}Sr, the amount of radioactivity administered must be kept low in order to reduce the radiation exposure to the patient, thus lengthy scanning times are required. Two alternatives have been proposed to the use of ^{85}Sr (120,90):

(a) using bone-seeking radiopharmaceuticals with shorter half-lives and

(b) more efficient instrumentation.

Larger doses of the short-lived radioisotopes could be given to the patient with less radiation exposure as compared to ^{85}Sr. Such doses would result in higher count rates so that the scan could be completed more rapidly (120). The use of the short-lived radioisotopes allows for sequential follow-up studies and permits other isotope studies within a relatively short time (90).

3. Strontium-87m

Strontium-87m was introduced by Meyers (122) as a substitute for ^{85}Sr. It can be milked from a generator system employing the 80 hour half-life ^{87}Y. Strontium-87m has a half-life of 2.8 hours and decays with the emission of a single gamma ray of 388 Kev to stable ^{87}Sr. Scans are performed 45 minutes after injection although about 50%-70% of the dose has not been taken up by bone

and remains in the circulation contributing somewhat to a lower tumor-to-background ratio (117). Administration of 250-1000 μCi, as the citrate or chloride, yields a radiation dose to the whole-body of 5-20 mRads and to the bone of 25-100 mRads. The major disadvantage of using ^{87m}Sr is the short half-life of the parent which necessitates the purchase of a new generator at least every two weeks (117).

4. Fluorine-18

Fluorine-18 has a very short half-life of 1.87 hours making its practical use feasible only in institutions in close proximity to a cyclotron or reactor from which it is produced. Fluorine-18 decays by the emission of a 650 Kev positron (87%) and the resulting 511 Kev annihilation gammas may be detected by coincidence techniques (121). After intravenous administration of ^{18}F, the fluoride ion is absorbed in the bone by anion exchange with the OH group in the hydroxyapatite at the surface of the bone crystal (117). This exchange occurs at sites of good blood supply and increased mineral turnover. About 50% of the injected ^{18}F is bound to bone with the remainder being rapidly excreted in the urine resulting in a high bone-to-background ratio (117). Scanning can commence about one hour after the injection (121). The administration of 1-2 mCi, as the sodium salt, results in a radiation dose to the whole-body of 35-70 mRads and to the bone of

120-360 mRads (121).

5. Radioisotopes of Gallium

The bone-seeking property of ^{72}Ga was described by Dudley (12,14). Gallium is thought to substitute for calcium in newly formed bone crystal (117).

Gallium-67 tends to localize in a variety of soft-tissue tumors as well as in tumors of the bone (36). Carrier-free ^{68}Ga failed to localize early in bone of test animals but when administered with carrier gallium, plasma protein-binding sites were saturated allowing a more rapid uptake of the ^{68}Ga into the bone (121). Administration of ^{68}Ga, as the citrate, in humans gives a whole-body radiation dose of 0.06 rads per mCi and a radiation dose to the bone of less than 0.364 rads per mCi (118).

C. The New Bone Scanning Agents

1. ^{99m}Tc-Polyphosphates

The excellent physical characteristics of ^{99m}Tc such as its short physical half-life of six hours, its monoenergetic gamma emission of 140 Kev and its ready availability from a generator system have contributed to its extensive use in Nuclear Medicine for imaging nearly every large organ in man (118). Recently, a ^{99m}Tc-polyphosphate has been investigated as a potential bone scanning agent (3). The intravenous administration of this compound to rabbits resulted in 37%-45% of the injected

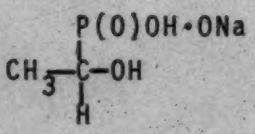
dose being localized in the bone between 1 and 24 hours after the injection. Blood concentration ranged from 13.72% after one hour to 4.37% after 24 hours. Cumulative urinary excretion after three hours was 45%-55% of the administered dose. A 10 mCi injection in humans was estimated to give a skeletal radiation dose of 0.45 rads, assuming that 50% of the injected radioactivity remained in the skeleton (3).

Technetium-99m complexes with polyphosphates of longer chain length and of higher molecular weight were investigated in an attempt to obtain faster blood clearances as compared to the original ^{99m}Tc-polyphosphate complex (123). A polyphosphate with an estimated chain length of 46 and a molecular weight of 4,660, as a ^{99m}Tc complex, was used for rabbit tissue distribution studies. Of the injected radioactivity, 43%-53% was localized in the skeleton between 1-24 hours after administration. The radioactivity in the blood was some three times lower than previously reported (3). A clinical study in a patient with known multiple skeletal metastases demonstrated excellent uptake of the complex in the involved areas of bone and the images were comparable to those obtained with ^{18}F in the same patient (123). A commercial preparation of the ^{99m}Tc-polyphosphate complex has since become available (124).

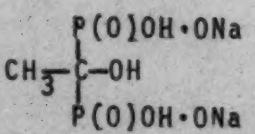
A number of polyphosphates have been compared for

their bone uptake in rats as a function of their molecular weight and chain length (125). A polyphosphate chain length of 40-60 groups and a molecular weight between 4,000-6,000 yielded the highest bone concentration and the best bone-to-nontarget tissue ratios. Polyphosphates with molecular weights in excess of 8,000 localized in the reticuloendothelial tissue and those below a molecular weight of 3,000 showed less deposition in bone and were rapidly cleared by the kidney.

2. Phosphonates and Diphosphonates



Phosphonate



Diphosphonate
(1,hydroxyethylidene-1,
1-disodiumphosphonate)
HEDSPA or EHDP

Castronovo et al. (126) studied the tissue distribution of ^{99m}Tc-HEDSPA in mice and a cumulative skeletal uptake of 55% of the injected dose was observed after three hours. The blood clearance of the complex was rapid with 4.53% of the injected dose in the blood after one hour and only 0.08% after six hours. Little radioactivity was detected in the remaining organs after three hours. The radiation dose to the skeleton was estimated to be 0.045 rads per mCi. It has been reported that

EHDP is resistant to chemical or enzymatic hydrolysis whereas the polyphosphates are believed to undergo enzymatic hydrolysis (127). ^{99m}Tc-EHDP has the reported advantage of a more rapid blood clearance and a relatively lower soft tissue concentration (127).

EXPERIMENTAL

I. Materials and Methods

A. Materials

All chemicals were of A.C.S. specification. Double distilled water was used throughout the entire study.

1. Anhydrous Gallium Trichloride ($GaCl_3$)

The $GaCl_3$ was purchased from Ventron Corporation, Alfa Products, Beverly, Massachusetts. A stock solution consisting of 1 g $GaCl_3$ per ml was prepared in 0.1N HCl to prevent the formation of $Ga(OH)_3$ (7). A working solution was adjusted to a concentration of 1.98 mg gallium per ml.

2. Sodium Tripolyphosphate ($Na_5P_3O_{10}$), Molecular Weight 367.93

This chemical was obtained in the form of purified granules from Fisher Scientific Company, Fair Lawn, New York, Lot Number 705245.

3. ^{14}C-EDTA [$(HOOC^{14}CH_2)_2NCH_2CH_2N(^{14}CH_2COOH)_2$]

Fifty microcuries of ^{14}C-EDTA was received from New England Nuclear, Boston, Massachusetts, having a specific activity of 3.42 mCi/mM (85.5 mg/mCi). An aqueous stock solution containing 5 µCi/ml was prepared.

4. ^{68}Ge-^{68}Ga Radioisotope Generator

The ^{68}Ge-^{68}Ga generator used throughout this investigation was obtained from New England Nuclear, Boston, Massachusetts. At the time of receipt, the expected ^{68}Ga radioactivity from the generator was stated to be 100 µCi. A

concentrated EDTA solution (Ethylenediaminetetraacetic Acid) was supplied with the generator. For elution, the EDTA was diluted to 1,000 ml with distilled water producing a concentration of 0.005M, the pH of which was adjusted to 7.0 with dilute NaOH. The final solution was stored in polyethylene containers, since glass can yield ions which decrease the EDTA titre (128).

5. Acids

Hydrochloric acid solutions (6N and 8N) were prepared from Reagent Grade HCl supplied by J.T. Baker Chemical Company, Phillipsburg, New Jersy. B.P. specifications were followed for the preparation of the acid solutions (129). Hydrochloric acid (0.1N) was prepared by dilution of Reagent Solution HCl obtained from B.D.H. Chemicals, Canada, Limited.

6. Bases

Sodium hydroxide (0.1N) was prepared from Reagent Solution NaOH purchased from B.D.H. Chemicals, Canada Limited. Sodium hydroxide (15N) was made according to B.P. specifications (129) from NaOH pellets, supplied by Allied Chemical, Canada Limited.

7. Ion-Exchange Resins

a. Rexyn-201

Rexyn-201, a certified analytical grade anion exchange resin, was purchased from Fisher Scientfic Company, Fair Lawn, New Jersy. This is a strongly basic organic anion exchanger consisting of polystyrene with alkyl

quaternary amine functional groups in the chloride-sulfate form. A medium porosity resin with a mesh size of 16-50 was used.

b. Dowex 1-X4

Dowex 1-X4 anion exchange resin was purchased from Bio-Rad Laboratories, Richmond, California. The resin is composed of polystyrene with quaternary ammonium functional groups in the chloride form. A large pore size and a mesh of 100-200 was used.

8. Animals

Adult male mice of the ALAS strain, weighing 20-30 g, were used for tissue distribution, excretion and toxicity studies. The mice were housed in a relatively stress-free environment in groups of six per cage with free access to food (Tekland Rockland mouse/rat diet) and water.

Rabbits used for tissue distribution studies were female of the New Zealand strain weighing 2.2-2.4 kg. Bone imaging studies were done on a female New Zealand rabbit weighing 3.8 kg and on a male rabbit of the Dutch strain weighing 1.96 kg.

Prior to use, all rabbits were individually caged with food and water available ad libitum.

B. Methods

1. Gamma Ray Spectrometry Techniques

Gallium-68 samples were assayed for radioactivity

using a Picker Autowell II gamma spectrometer, (Picker Nuclear Corporation, White Plains, New York). The 0.511 Mev photopeak counting efficiency for this spectrometer, as calculated using a ^{22}Na standard, was approximately 21%. Due to the short half-life of ^{68}Ga, counting times were limited to one minute per sample.

2. Liquid Scintillation Spectrometry

Carbon-14 containing samples were assayed for radioactivity in a liquid scintillation spectrometer (Liquimat 200, Picker Nuclear Corporation, White Plains, New York). Samples were mixed with 10 ml of Aquasol scintillant (New England Nuclear, Boston, Massachusetts). Quench corrections were effected by the isotope channels-ratio method employing a set of ^{14}C quenched standards prepared by the addition of various quantities of 8N HCl.

3. Statistical Methods and Computer Programs

All formulae and computer programs used throughout this investigation are presented in Appendixes 2 and 3. A digital PDP8/L computer (Digital Equipment Corporation, Maynard, Massachusetts) was used for data processing.

4. Chromatographic Techniques

A modification of the solvent system described by Poonia *et al.* (130) consisting of chloroform-acetone-isoamyl alcohol (1:1.5:1) was used to separate the $^{68}GaCl_3$ from ^{68}Ga-EDTA on Chromar-500 chromatographic paper (Mal-

linckrodt Chemical Works, Laboratory Products, Montreal, Canada). A solvent system consisting of 95% ethanol-water (1:1) was used for distinguishing between $^{68}Ga(OH)_3$ and ^{68}Ga-Polyphosphate on Whatman No. 1 filter paper.

5. Imaging Procedures

A Pho/Gamma-Positron III Scintillation Camera (Nuclear Chicago Corporation, Des Plains Illinois) was used for bone imaging. Maximum coincidence counting rates were obtained with a detector spacing of 18 inches. Two planes of "best focus" were simultaneously viewed and electronically varied in order to find the focal plane setting that most nearly coincided with the localization of radioactivity within the rabbit. Images were recorded on polaroid film after an accumulated count of 17-100 k had been reached.

6. Quality Control of the ^{68}Ge-^{68}Ga Generator

The ^{68}Ge-^{68}Ga generator used throughout this study was previously described by Greene (5) and is illustrated in Figure 1.

a. Confirmation of Manufacturer's Specifications

The instructions accompanying the ^{68}Ge-^{68}Ga generator stated that elution of the generator with 25 ml of 0.005M EDTA would be complete within four minutes. However, the actual elution time was much longer, being 22 minutes for 25 ml of EDTA and 12.5 minutes for 15 ml of EDTA. Aliquots of these two EDTA elution volumes were assayed for

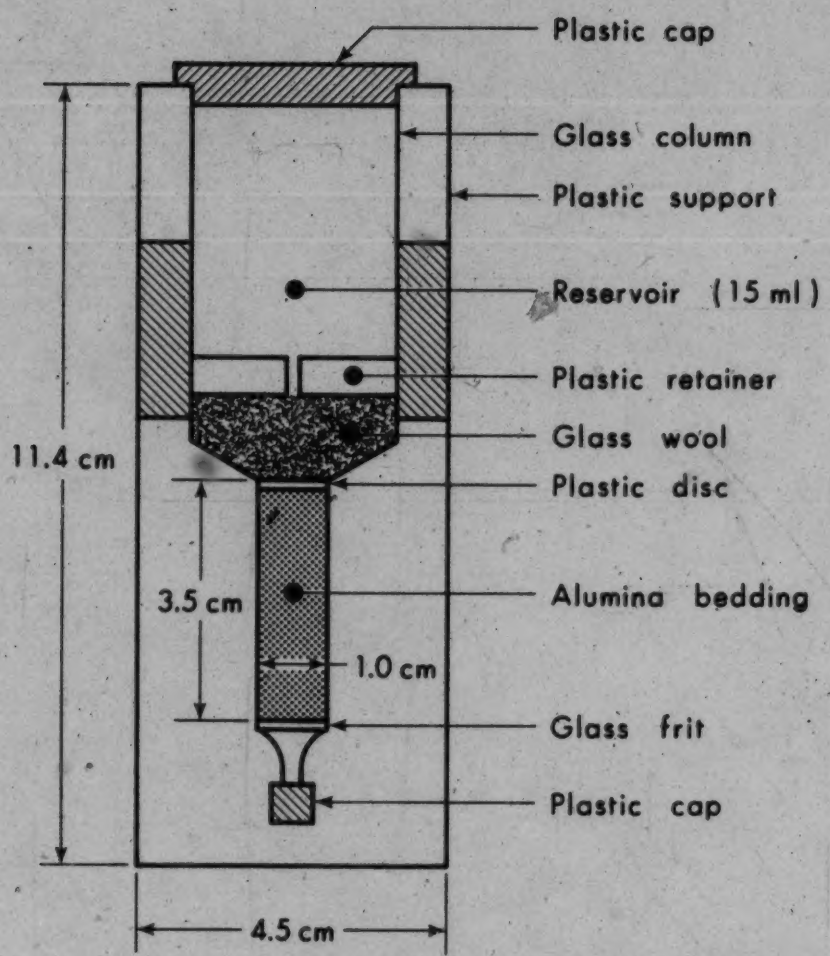


Figure 1

The ^{68}Ge-^{68}Ga Radioisotope Generator

radioactivity in the gamma spectrometer. From the previously determined spectrometer efficiency, the total eluted radioactivity was calculated to be about 98 μCi with 25 ml of EDTA and 95.5 μCi with 15 ml of EDTA.

Since the total amount of ^{68}Ga obtained with each of these EDTA volumes was nearly the same, all future elutions utilized 15 ml of EDTA solution.

b. Radionuclidic Purity

The energy spectrum of the ^{68}Ga-EDTA eluate was determined by differential pulse-height analysis using a NaI(Tl) detector, (Picker Nuclear Corporation, White Plains, New York) as well as a Ge(Li) detector (Nuclear Diodes, Prairie View, Illinois). The energy spectrum from the latter was stored in a multichannel analyzer (Northern Scientific, Middleton, Wisconsin) and recorded on an x - y plotter.

The peaks observed in Figures 2 and 3 can be attributed to gamma rays from ^{68}Ga which arises from and is in equilibrium with any ^{68}Ge impurity present in the eluate. These results are consistent with previously published data (131).

c. Determination of the ^{68}Ge Leakage

The level of ^{68}Ge contamination in the ^{68}Ge-^{68}Ga-generator eluate was estimated by counting an aliquot of the eluate in the gamma spectrometer for a total accumulated count of 200,000 counts or for a total counting time of one hour, whichever came first. An empty sample tube was handled in the same manner to provide a background count. The

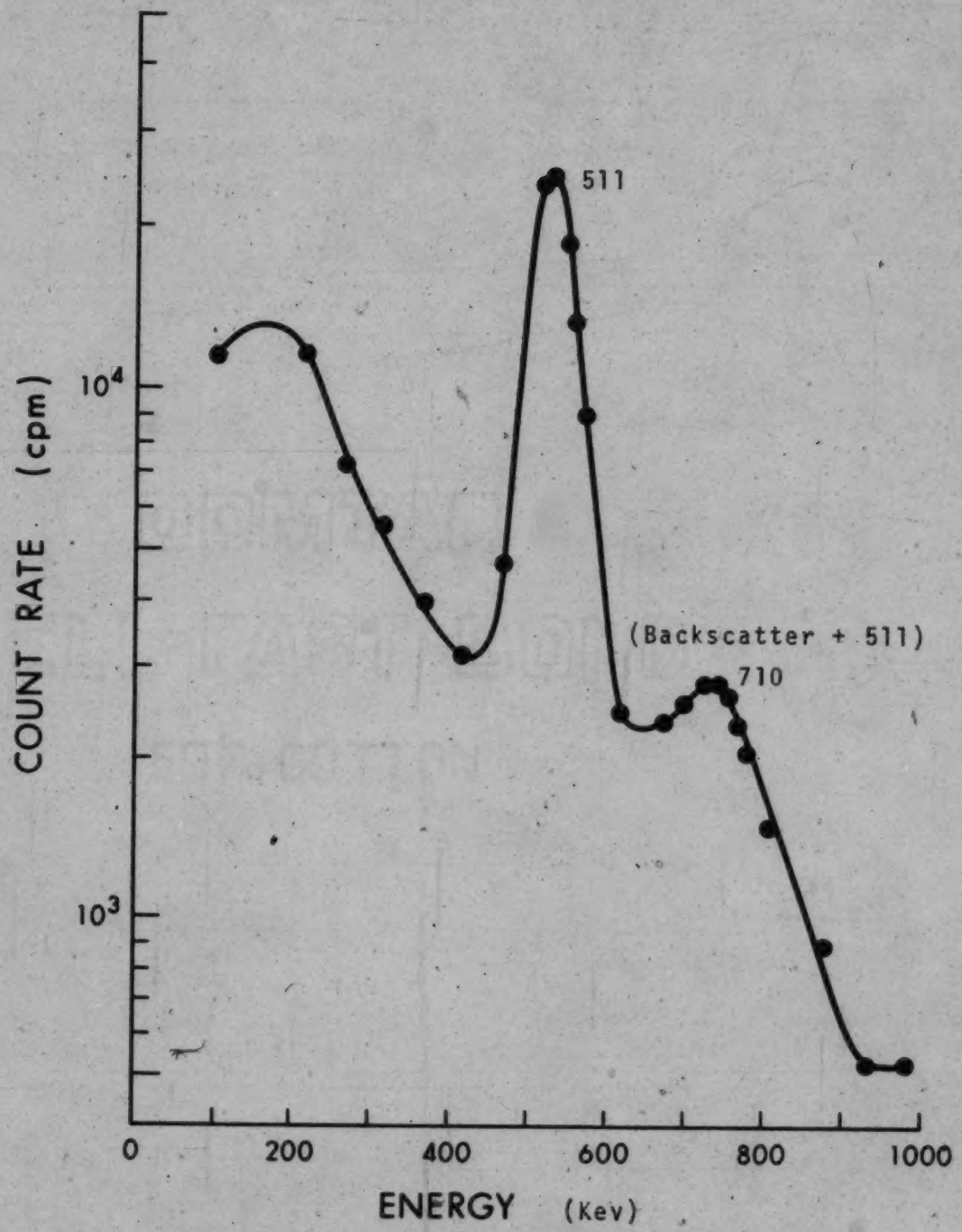


Figure 2

Energy Spectrum of Gallium-68 Determined
Using a NaI(Tl) Detector

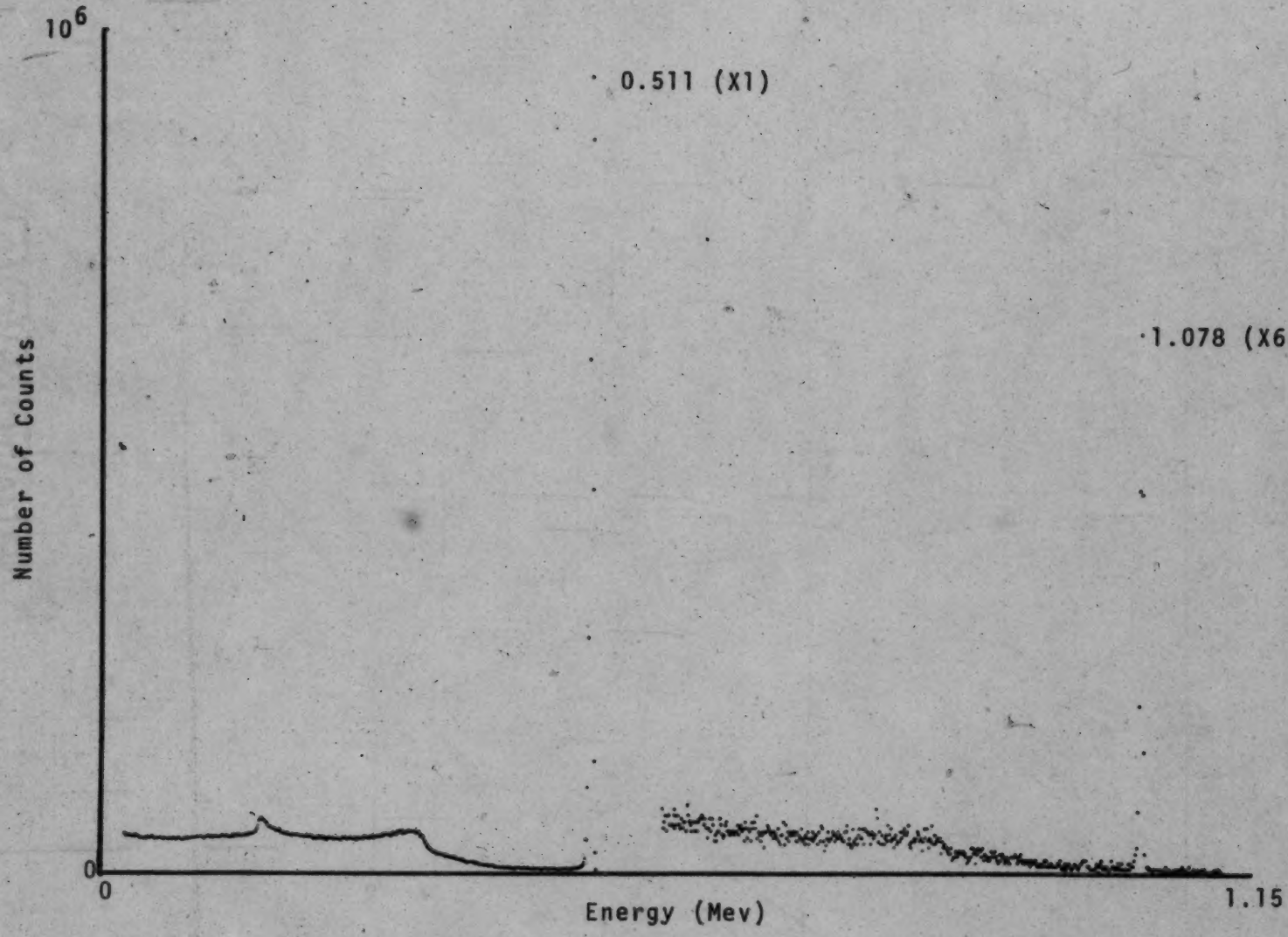


Figure 3

Energy Spectrum of Gallium-68 Determined Using a Ge(Li) Detector

tubes were repeatedly counted for a 24-48 hour period. It was assumed that any radioactivity in the eluate (above background levels) after this time period would be due to the presence of the long-lived parent. Computer program A and Equation 1 were used to calculate a ratio comparing the ^{68}Ge radioactivity to that of the ^{68}Ga radioactivity present at the time of elution of the generator. Computer program A was also used to plot the parent-daughter decay curve as shown in Figure 4. The above procedure was repeated after a various number of elutions and the results are summarized in Table VII.

TABLE VII

^{68}Ge Leakage in ^{68}Ga Eluate

Elution Number	Leakage[(a)]
1	3.75×10^{-4}%
2	1.44×10^{-4}%
9	3.72×10^{-5}%

(a) Expressed as % of the ^{68}Ga radioactivity at time of elution of the generator

The ^{68}Ge contamination of the generator eluate decreased as the number of elutions increased, in agreement with earlier reports in the literature (89).

d. Determination of the Recovery Time of the ^{68}Ga-Generator

In order to obtain maximum amounts of ^{68}Ga with

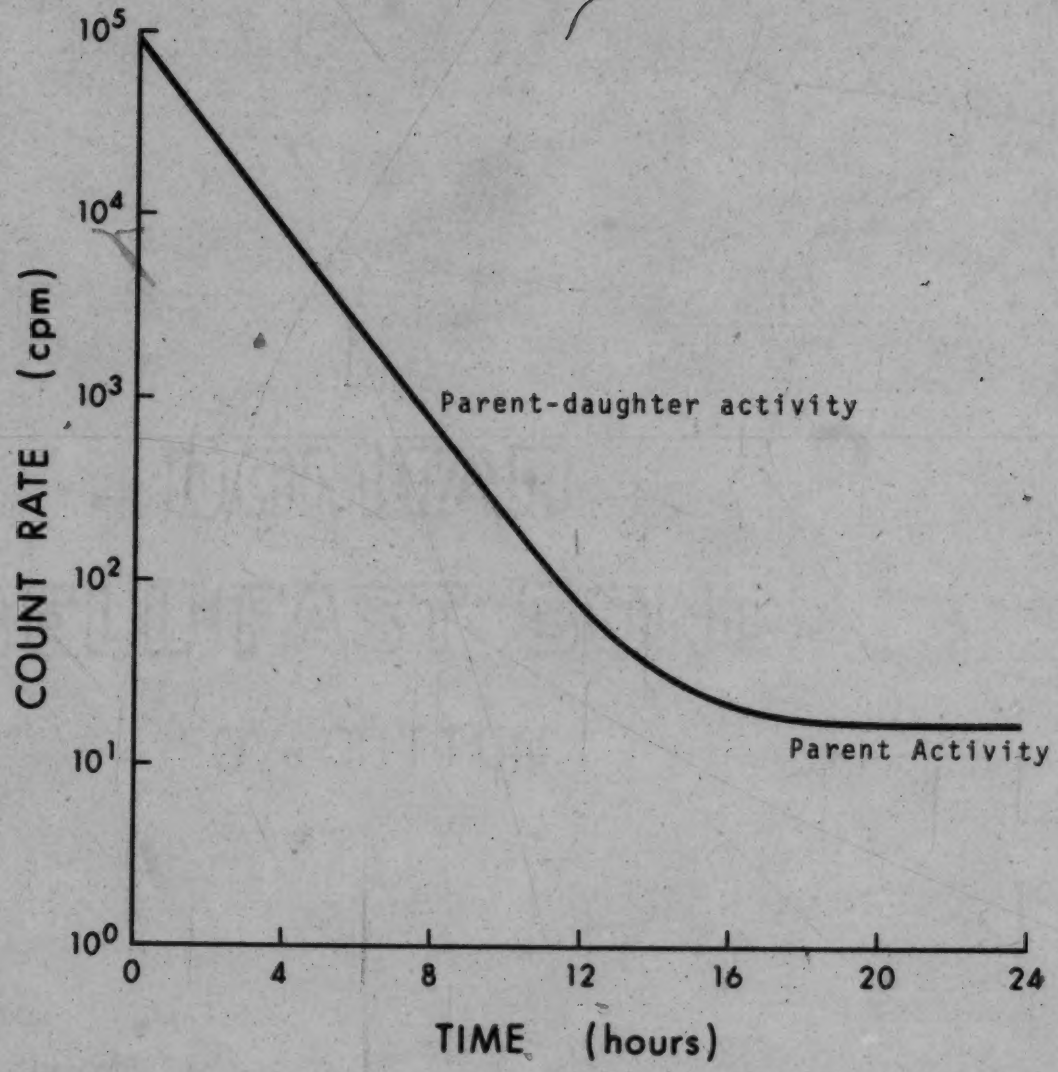


Figure 4

Parent-Daughter Decay Curve

each subsequent elution of the generator the following experiment was performed: On the first day of each week the generator was milked twice. This was repeated over three consecutive weeks allowing different recovery periods between the two elutions, as shown in Table VIII. The results in Table VIII indicated that about three hours were required for the generator to attain 93.5% of the previously eluted radioactivity. It has been reported that the ^{68}Ge-^{68}Ga-generator could be eluted every three to four hours (2),

e. Characteristics of the Generator Eluate

The following experiment was performed to determine which portion of the generator eluate contained the greatest amount of radioactivity. The generator eluate was collected serially into a number of sample tubes and assayed for ^{68}Ga radioactivity in the gamma spectrometer. The results, as calculated using computer program B, are shown in Table IX.

An eluate volume of 7.0-7.5 ml contained approximately 85% of the total eluted radioactivity. Therefore, such volumes were used consistently throughout the entire study so that a ^{68}Ga sample of high specific activity could be obtained.

7. Preparation of the ^{68}Ga-Polyphosphate

Prior to preparing the ^{68}Ga-polyphosphate complex it was first necessary to dissociate the ^{68}Ga-EDTA and to separate the two components. Various procedures have been

TABLE VIII

Determination of the Recovery Time for the ^{68}Ge-^{68}Ga-Generator

Week	Radioactivity (cpm) in First Elution (A)	Elapsed Time After First Elution (hrs)	Radioactivity (cpm) in Second Elution (B)	Ratio B/A
1	305594	1	164071	.536
2	264491	2	209621	.793
3	248881	3	232607	.935

TABLE IX

Gallium-68 Radioactivity in Various Portions of the ^{68}Ge-^{68}Ga Generator Eluate

Sample No.	Cumulative Volume Collected (ml)	Cumulative Radioactivity Eluted (μCi)	Percent of Total Radioactivity Eluted
1	0.68	0.02	0.02
2	1.41	1.05	1.44
3	2.12	24.94	34.39
4	2.79	42.51	58.63
5	3.52	49.79	68.67
6	4.16	52.86	72.90
7	4.85	55.21	76.14
8	5.45	57.05	78.68
9	6.07	58.73	80.99
10	6.71	60.26	83.11
11	7.28	61.58	84.93
12	7.86	62.79	86.59
13	8.44	63.87	88.08
14	9.00	64.87	89.46
15	9.52	65.65	90.54
16	10.06	66.54	91.76
17	10.58	67.38	92.93
18	11.14	68.27	94.15
19	11.64	69.00	95.16
20	12.13	69.74	96.18
21	12.58	70.38	97.96
22	13.09	71.13	98.09
23	13.50	71.71	98.89
24	13.96	72.34	99.76
25	14.08	72.51	100.00

reported (51,77,90,91) but a modification of the method as described by Carlton (91) appeared to be the most applicable due to its rapidity and efficiency. The method involved the dissociation of the ^{68}Ga-EDTA complex with concentrated HCl followed by ion-exchange separation of the two components (132). The ^{68}Ga, as $^{68}GaCl_4^-$ was retained by the resin while the EDTA passed through the resin bed with the concentrated HCl (7). The ^{68}Ga was subsequently removed from the resin as $^{68}GaCl_3$ by the addition of dilute HCl.

a. Dissociation and Separation of the ^{68}Ga-EDTA

The ion-exchange resin was washed thoroughly with water and decanted to remove any fine particles. A slurry of the ion-exchange resin in 8N HCl was poured into a buret of 1.1 cm inside diameter and packed to a height of 3.5 cm. The following procedure was then adopted for the dissociation of the chelate and the separation of its components.

(i) 7 ml of generator eluate were mixed with 7 ml of 8N HCl

(ii) the mixture was applied to the top of the resin column and the flow rate was adjusted to about 0.5 ml/minute

(iii) 1 ml portions of the eluate were collected into counting tubes

(iv) after all of the original mixture had passed through the resin, an additional 10 ml of 8N HCl were added to the column to remove any remaining EDTA; 1 ml portions of eluate were also collected

2 2

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(v) the ^{68}Ga was removed from the resin by the addition of 10 ml of 0.1N HCl, and 1 ml portions of eluate were collected

(vi) all the sample tubes were then assayed for ^{68}Ga radioactivity in the gamma spectrometer

(vii) all relative calculations were done using computer program C (Appendix 3).

With the Rexyn-201 resin, about 40% of the available radioactivity was recovered in the 0.1N HCl fraction, whereas with the Dowex 1-X4 resin, about 96% recovery was obtained. It was also observed that the first 2-3 ml of the 0.1N HCl fraction collected off the resin contained essentially no radioactivity since this represented the bed volume of the resin. However, the next 3-4 ml contained about 98% of the available ^{68}Ga. Therefore, in all future separation procedures, the first 3 ml of the 0.1N HCl fraction were discarded and the next 3 ml were collected.

b. Efficiency of the Dissociation and Ion-Exchange Separation Procedures

A paper chromatographic technique was designed in an effort to determine the efficiency of the dissociation procedure. The solvent system chloroform-acetone-isoamyl alcohol (1:1.5:1) and Chromar-500 chromatographic paper were used. A sample of the generator eluate remained at the origin, whereas a sample of the eluate after the dissociation and separation procedures migrated with an Rf value of 0.7.

These results indicated that the ^{68}Ga-EDTA complex was dissociated yielding $^{68}GaCl_3$ in the 0.1N HCl fraction. However, the possibility of the presence of some free EDTA in the 0.1N HCl was also considered. For this reason a quantitative estimate of the amount of EDTA in the 0.1N HCl fraction was made.

Initially the colorimetric method for estimating EDTA as described by Brady and Gwilt (133) was used but proved unsatisfactory probably due to the presence of interfering cations. Therefore, an experiment using ^{14}C-EDTA as a tracer was designed, as outlined below:

(i) a 0.1 ml aliquot of the ^{14}C-EDTA solution containing 5 µCi was thoroughly mixed with 7 ml of generator eluate in 7 ml of 8N HCl

(ii) this mixture was applied to the top of a Dowex 1-X4 resin column

(iii) 25 ml of the 8N HCl eluate, (fraction A) and 8 ml of the 0.1N HCl eluate (fraction B) were collected

(iv) four 1 ml aliquots from both fractions were transfered into liquid scintillation vials containing 10 ml of Aquasol

(v) the eight vials were set aside for 48 hours to allow for the decay of the ^{68}Ga radioactivity, after which time the vials were assayed for ^{14}C radioactivity in the liquid scintillation.

spectrometer. A vial containing fluor only was used to provide a background count. The results are summarized in Table X.

TABLE X

^{14}C-EDTA Radioactivity in Dowex 1-X4 Eluate

Number of samples from each fraction	:	4
Radioactivity (fraction A)	:	835969 ± 18265 dpm/ml
Radioactivity (fraction B)	:	45 ± 3.36 dpm/ml
Background (average of 10 determinations)	:	39.5 ± 3.75 cpm

The radioactivity in fraction B was much lower than that in fraction A. However, the significance of the difference between the count rates of fraction B and background was not immediately apparent. The following experiment was performed to determine if the difference observed was significant.

(i) each of the four aliquots representing fraction B were recounted a total of ten times

(ii) the mean count rate and its standard deviation were calculated for each aliquot

(iii) a Student t test was used to compare the mean and standard deviation of each sample to that of the background.

At the 95% confidence level, the difference between sample and background count rates were found to be significant.

The net count rate for each of the four samples was calculated using Equation 3 (Appendix 2). The average net count rate of the four samples as determined by Equation 4 (Appendix 2) was 9.5 ± 5.4 dpm/ml or 75.2 ± 43.28 dpm/8 ml. This represented 0.00865 ± 0.0049% of the ^{14}C radioactivity initially added to the Dowex 1-X4 resin. Since 9.535 mg EDTA (both unlabeled and as ^{14}C labeled) had been initially added to the Dowex 1-X4 resin, the quantity of EDTA in the 0.1N HCl fraction was therefore estimated to be 0.825 ± 0.467 µg. This amount of EDTA was comparable to that reported by Carlton (91) who used the colorimetric method and found an EDTA content of 5 µg in the 0.1N HCl fraction.

8. Preparation of Compounds for Animal Studies

The addition of carrier gallium to a ^{68}Ga-citrate preparation has been shown to cause enhanced bone uptake of the ^{68}Ga due to saturation of the plasma protein-binding sites (41). The formation of $Ga(OH)_3$ from $GaCl_3$ at physiological pH or by the addition of NaOH to a $GaCl_3$ solution has also been established (7). In addition, $Ga(OH)_3$ can partially exist in a colloidal form and as such could be taken up in the liver, spleen and bone marrow. For these reasons, tissue distribution studies in mice were done in order to compare and evaluate the tissue uptake characteristics of ^{68}Ga-polyphosphate, ^{68}Ga-polyphosphate plus carrier gallium (hereafter referred to as ^{68}Ga-gallium-polyphosphate) and $^{68}Ga(OH)_3$. The uptake of ^{68}Ga-gallium-polyphosphate and

$^{68}Ga(OH)_3$ was also studied using rabbits in order to compare the uptake in bone and bone marrow of these two compounds.

a. Preparation of ^{68}Ga-Polyphosphate

Based on previously reported results, (3), a polyphosphate dose of 5 mg/kg body weight was used throughout the tissue distribution studies. The ^{68}Ga-polyphosphate was prepared as follows:

(i) 3 ml of $^{68}GaCl_3$ in 0.1N HCl were collected from the Dowex 1-X4 resin

(ii) a filtered sodium tripolyphosphate solution, sufficient to give a polyphosphate dose of 5 mg/kg was added to the $^{68}GaCl_3$; the solution was mixed for two to three minutes on a magnetic stirrer

(iii) the pH was adjusted to 7.0 first by the addition of a few drops of 15N NaOH followed by a few drops of 0.1N NaOH

(iv) the final volume was adjusted to 5.0 ml with distilled water

(v) if necessary, the pH was readjusted to 7.0 with 0.1N NaOH

(vi) the final solution was mixed for an additional two to three minutes before injecting into the test animals.

b. Preparation of ^{68}Ga-Gallium-Polyphosphate

Preliminary animal studies following the administration of ^{68}Ga-gallium-polyphosphate in mice, at a dose of

1.98 mg gallium (5 mg $GaCl_3$) per kg, resulted in a high liver uptake as compared to that of ^{68}Ga-polyphosphate. When 0.98 mg (2.5 mg $GaCl_3$) of gallium/kg was added as a carrier, this high uptake in the liver was not seen. The ^{68}Ga-gallium-polyphosphate was prepared as follows:

(i) 3 ml of $^{68}GaCl_3$ in 0.1N HCl were collected from the Dowex 1-X4 resin

(ii) a filtered sodium tripolypnosphate solution, sufficient to give a polyphosphate dose of 5 mg/kg was added to the $^{68}GaCl_3$; the solution was mixed for two to three minutes on a magnetic stirrer

(iii) a sufficient amount of $GaCl_3$ was added to the above solution to give a dose of 0.98 mg gallium/kg; the solution was again mixed for two to three minutes

(iv) the pH was then adjusted to 7.0 by first adding a few drops of 15N NaOH followed by the addition of one to two drops of 0.1N NaOH

(v) the final volume was adjusted to 5 ml with distilled water

(vi) if necessary, the pH was readjusted to 7.0 with 0.1N NaOH

(vii) the final solution was mixed for an additional two to three minutes before injecting into the test animals.

c. Preparation of $^{68}Ga(OH)_3$ Solution

The method used for the preparation of this compound was as follows:

(i) 3 ml of $^{68}GaCl_3$ in 0.1N HCl were collected from the Dowex 1-X4 resin

(ii) the pH was adjusted to 7.0, first by the addition of a few drops of 1N NaOH followed by one to two drops of 0.1N NaOH

(iii) the final volume was adjusted to 5 ml with distilled water

(iv) if necessary, the pH was readjusted to 7.0 with 0.1N NaOH

(v) the final solution was mixed for an additional two to three minutes before injecting into the test animals.

II. Animal Studies

A. Tissue Distribution in Mice

The various compounds of ^{68}Ga were slowly injected in mice *via* the tail vein. The volumes injected contained 1-2 μCi of radioactivity and did not exceed 0.25 ml. After injection the animals were kept in metabolism cages and the urine and feces were collected separately. Food and water were available *ad libitum* throughout the postinjection period. At specified time intervals the mice were sacrificed by decapitation. A blood sample was collected in a heparinized

syringe and a 0.2 ml aliquot was transfered to a counting tube. The tissues removed for assay included the lungs, liver, G.I.T. and contents (referred to hereafter as G.I.T.), spleen, kidney, brain, muscle, heart and bone (femur and tibia). All tissue samples were rinsed clean of excess blood in distilled water, blotted dry, weighed and placed into counting tubes. Each sample was assayed for ^{68}Ga radioactivity in the gamma spectrometer. The tail, remainder of the corpse, urine and feces were also counted in order to calculate the total radioactivity recovered.

Computer program C was used to calculate the radioactivity in each tissue sample as well as to calculate the percentage of the total administered radioactivity which was recovered in each tissue. The total radioactivity injected was calculated by counting an aliquot of the original preparation equivalent to the volume injected. All sample counts were corrected for background and for radioactive decay to the time of elution of the generator.

B. Tissue Distribution in Rabbits

For the rabbit tissue distribution studies, 5-10 μCi of ^{68}Ga radioactivity were injected into the marginal ear vein in a volume of 5-6 ml. After injection the rabbits were individually kept in cages with free excess to food and water. Three hours after the injection, a blood sample was collected in a heparinized syringe by cardiac puncture

and a 1 ml aliquot was transferred to a counting tube. The rabbits were then sacrificed and the tibia and femur from one leg were removed and cleaned of all adhering tissue. The marrow from each of these bones was collected, weighed and transfered into counting tubes. The bones were washed, cut into small pieces, weighed and distributed into a series of counting tubes. Samples of liver, lung, spleen, kidney and muscle were also obtained. All samples were assayed for ^{68}Ga radioactivity in the gamma spectrometer. Computer program C was used to calculate the radioactivity in each sample as well as the percentage of the total administered radioactivity which was recovered in each sample. All values were corrected for background and for radioactive decay to the time of elution of the generator.

C. Toxicity Studies

The toxicities in mice of solutions of sodium tripolyphosphate and of sodium tripolyphosphate containing carrier gallium were investigated. Various concentrations of these two preparations were administered by intravenous injection to groups of mice.

The mice were observed over a postinjection period up to 30 days. During this period the mice were weighed on various days and their weights were compared to those of control animals.

At postinjection time intervals of 7 and 30 days,

tissue samples of lung, liver and kidneys were excised from test and control animals and preserved in a 10% formaldehyde solution for histopathological examination. Bone tissue samples of the rib, humerus, and femur were obtained from test and control mice 30 days after the injection for histopathological studies.

D. Imaging

Rabbits used for bone imaging studies were tranquilized by the administration of an intramuscular dose of 0.15 ml/kg body weight of Innovar-Vet (McNeil Laboratories, Don Mills, Ontario). Approximately 10 μCi of either ^{68}Ga-polyphosphate or ^{68}Ga-gallium-polyphosphate were administered *via* the marginal ear vein in a volume of 5-6 ml. The rabbits were positioned between the two detectors of the Pho/Gamma-Positron III Camera. A series of images were recorded beginning approximately 17 minutes after the administration of the compounds.

RESULTS AND DISCUSSION

I. Characteristics of the ^{68}Ga-Polyphosphate Complex

The procedure described for the preparation of ^{68}Ga-polyphosphate in this study was based on the results of a number of preliminary experiments. Initially, a 0.005M sodium tripolyphosphate solution was used to elute the generator in an attempt to form the ^{68}Ga-polyphosphate complex directly. This method was unsuccessful in that the total radioactivity eluted using the sodium tripolyphosphate solution dropped from an initial level of about 42 µCi to about 1.6 µCi after several elutions. However, when 0.005M EDTA solution was again used, the radioactivity in the eluate was restored to normal levels. Elution of the generator with water produced similar low levels of ^{68}Ga as was obtained with the sodium tripolyphosphate solution. From these results it was concluded that elution of the generator with a sodium tripolyphosphate solution was not feasible. Thus, the procedures involving dissociation of the ^{68}Ga-EDTA and the ion-exchange separation of the two components was used to obtain $^{68}GaCl_3$ as a starting material for the preparation of the ^{68}Ga-polyphosphate complex.

When 500 mg of stable $GaCl_3$ was added to a solution containing 2 g of sodium tripolyphosphate, a cloudy suspension formed which cleared after the solution was mixed. It has been reported that polyphosphates form insoluble salts with polyvalent metal ions and that these salts can be dissolved by the formation of soluble complexes in the

presence of excess polyphosphate (85). Based on this observation, it was suspected that a Ga-polyphosphate complex had been formed as noted by the disappearance of the cloudiness in the solution.

Further substantiation of this complex formation was attempted using paper chromatography and the solvent system consisting of chloroform-acetone-isoamyl alcohol (1:1.5:1) on Chromar-500 paper to separate $^{68}GaCl_3$ and ^{68}Ga-polyphosphate. A sample of the mixture when applied to the paper showed that ^{68}Ga-polyphosphate remained at the spot of origin while the $^{68}GaCl_3$ migrated with an Rf value of 0.7. However, a neutralized $^{68}GaCl_3$ solution also remained at the origin, probably as the $Ga(OH)_3$ (7). Thus, by this method, it was not possible to conclusively distinguish between a ^{68}Ga-polyphosphate complex at pH 7.0 and $^{68}Ga(OH)_3$ at pH 7.0. It was also noted that the addition of 6N NaOH to a solution containing 250 mg of $GaCl_3$ produced a precipitate at pH 7.0, probably as $Ga(OH)_3$ (7). Addition of 6N NaOH to the same solution containing 1 g of sodium tripolyphosphate failed to produce a precipitate. This could have indicated that a Ga-polyphosphate complex had been formed in the latter case, thereby preventing the formation and precipitation of $Ga(OH)_3$. This $Ga(OH)_3$ suspension, when added to a sodium tripolyphosphate solution, failed to produce the clear solution as was previously noted for $GaCl_3$ and sodium tripolyphosphate.

Since aqueous polyphosphate solutions can be precipitated by the addition of alcohol or acetone (96), infrared spectrometric techniques were employed in an effort to distinguish between a sodium tripolyphosphate solution and a Ga-polyphosphate complex. Ethanol was added to a solution containing 20 g of sodium tripolyphosphate as well as to a similar sodium tripolyphosphate solution containing 500 mg of $GaCl_3$. In both cases a precipitate formed upon the addition of 10 ml of ethanol. The I.R. spectra obtained for these two precipitates using both the KBr pellet and nujol mull methods on a Unicam SP 1000 Infrared Spectrophotometer (Pye Unicam Limited, Cambridge, England) were identical. Thus, it was not possible to differentiate between these two compounds by this technique.

Complex formation was subsequently checked using paper chromatography and the solvent system 95% ethanol-water (1:1). Using Whatman No. 1 filter paper, a sample of the ^{68}Ga-polyphosphate migrated with an Rf of 0.6, but a sample of $^{68}Ga(OH)_3$ remained at the origin.

From the above series of results, it was concluded that:

(i) ^{68}Ga-polyphosphate cannot be eluted from a ^{68}Ge-^{68}Ga generator using sodium tripolyphosphate

(ii) ^{68}Ga-polyphosphate could be formed by thoroughly mixing a solution of $^{68}GaCl_3$ with an excess of sodium tripolyphosphate

(iii) in the absence of sodium tripolyphosphate or failure of the complex to be formed, $^{68}Ga(OH)_3$ will form at pH 7.0

(iv) the solvent system of 95% ethanol-water (1:1) can be used to distinguish between $^{68}Ga(OH)_3$ and ^{68}Ga-polyphosphate

(v) $^{68}Ga(OH)_3$, when mixed with a sodium tripolyphosphate solution, will not form a ^{68}Ga-polyphosphate complex

II. Tissue Distribution in Mice

The tissue distribution studies following the intravenous administration of the various ^{68}Ga-radiopharmaceuticals in mice were performed on a minimum of five mice at each of the various time intervals. Because of the short physical half-life of the ^{68}Ga radioisotope and also the low levels of radioactivity that were available for injection (approximately 1.0-2.0 μCi per injected volume), the distribution studies were extended to a maximum of six hours only. The results of the tissue distribution studies were expressed as a percentage of the injected dose per gram of tissue and as a percentage of the injected dose per total organ. In the latter case, it was assumed that the bone, blood, muscle and bone marrow represented 10%, 7%, 43% and 2.2% of the total body weight respectively (3,126).

A. ^{68}Ga-Polyphosphate Tissue Distribution Studies

Table XI shows that soon after the administration of the ^{68}Ga-polyphosphate complex, the blood and the lungs exhibited the greatest concentration of radioactivity which slowly decreased throughout the various time intervals. Since gallium is known to bind to plasma proteins, specifically, transferrin (34,35), this high level of radioactivity in the blood was therefore probably due to protein-binding of the ^{68}Ga. As represented in Figure 5, the levels of radioactivity in the lungs and blood were nearly parallel. The concentration of radioactivity in each of these organs decreased as time elapsed. Because of this similarity in behavior, it was assumed that the majority of the radioactivity in the lungs was due to the radioactivity of the blood within the lung. It would also appear that as the ^{68}Ga-polyphosphate complex left the blood compartment, it became available for bone uptake which increased throughout the period of study. The bone uptake reached a maximum after three to four hours which compares to ^{99m}Tc-STPP which requires a delay of three to four hours prior to the start of an imaging procedure (3).

Table XII and Figure 6 show that the muscle and G.I.T. localized an appreciable amount of the ^{68}Ga-polyphosphate complex. The radioactivity in the G.I.T. could not be ascribed to excretion of the complex in the feces as only 2.5% of the administered radioactivity was recovered

TABLE XI

Tissue Concentration of Radioactivity After Intravenous Administration of ^{68}Ga-Polyphosphate in Mice[a,b]

Tissue	Time After Administration			
	15 Minutes	30 Minutes	1 Hour	2 Hours
Bone	7.49 ± 1.43	6.77 ± 0.96	8.51 ± 1.32	11.77 ± 0.89
Brain	0.76 ± 0.19	0.45 ± 0.09	0.27 ± 0.17	0.33 ± 0.21
Lung	15.92 ± 2.25	13.57 ± 3.48	12.25 ± 0.39	8.94 ± 2.61
G.I.T.	2.14 ± 0.25	2.43 ± 0.14	3.09 ± 0.54	3.77 ± 0.13
Heart	5.69 ± 1.35	5.16 ± 0.81	4.45 ± 0.69	3.63 ± 0.72
Spleen	4.87 ± 1.47	4.78 ± 1.03	3.93 ± 1.57	3.59 ± 0.80
Kidney	4.20 ± 1.13	4.67 ± 0.89	4.82 ± 0.75	5.69 ± 1.49
Liver	3.67 ± 0.79	3.59 ± 0.73	3.11 ± 0.75	3.58 ± 0.62
Blood[c]	21.17 ± 5.74	16.85 ± 2.71	12.14 ± 2.11	9.94 ± 1.00
Muscle	1.87 ± 0.19	1.76 ± 0.20	1.74 ± 0.28	1.62 ± 0.19

...continued

TABLE XI (continued)

Tissue	Time After Administration		
	3 Hours	4 Hours	6 Hours
Bone	14.74 ± 3.11	15.46 ± 2.57	19.06 ± 3.14
Brain	0.32 ± 0.14	0.32 ± 0.15	0.49 ± 0.31
Lung	10.24 ± 2.78	8.24 ± 0.95	7.95 ± 0.84
G.I.T.	4.96 ± 0.82	4.77 ± 0.99	5.48 ± 1.81
Heart	4.42 ± 1.13	3.59 ± 1.65	2.98 ± 2.36
Spleen	4.48 ± 1.12	3.70 ± 1.67	5.54 ± 1.92
Kidney	5.47 ± 0.66	5.28 ± 0.77	6.41 ± 2.35
Liver	3.78 ± 0.53	3.48 ± 0.52	4.43 ± 0.79
Blood	9.69 ± 2.23	8.41 ± 1.03	8.69 ± 1.74
Muscle	1.79 ± 0.24	2.17 ± 0.46	1.82 ± 1.07

[a] Percent of injected radioactivity per gram of tissue

[b] Mean of five animals ± standard deviation

[c] Percent of injected radioactivity per milliliter of blood

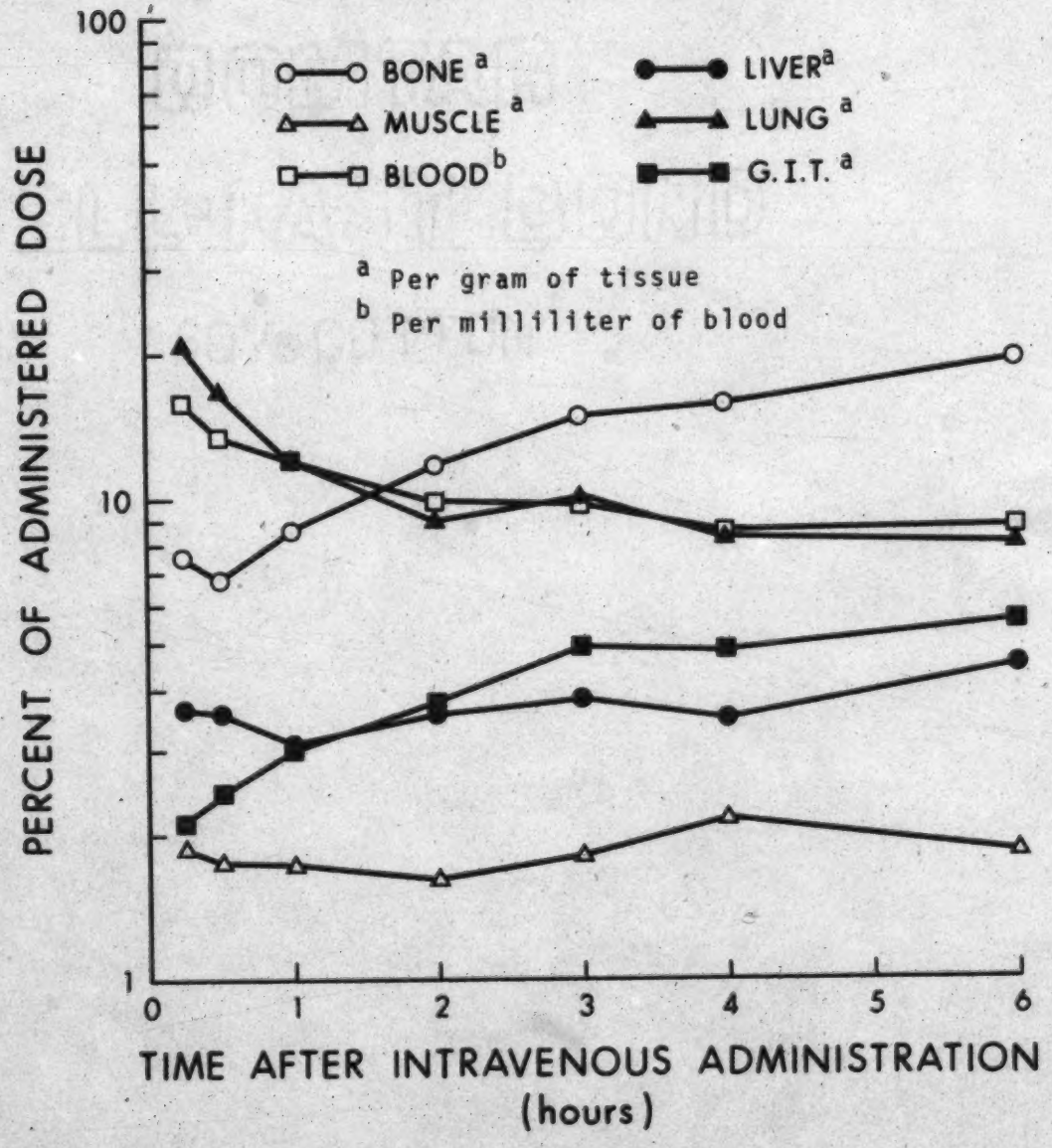

Figure 5

Tissue Distribution of ^{68}Ga-Polyphosphate in Mice

TABLE XII

Whole Organ Uptake of ^{68}Ga-Polyphosphate After Intravenous Administration in Mice[a,b]

Tissue	Time After Administration			
	15 Minutes	30 Minutes	1 Hour	2 Hours
Bone	23.22 ± 3.44	21.19 ± 3.45	25.73 ± 3.64	33.46 ± 1.61
Brain	0.32 ± 0.07	0.19 ± 0.03	0.12 ± 0.08	0.15 ± 0.09
Lung	3.82 ± 1.39	3.34 ± 1.22	2.99 ± 0.46	2.57 ± 0.68
G.I.T.	9.02 ± 0.39	10.14 ± 0.66	11.94 ± 1.32	14.66 ± 1.35
Heart	0.62 ± 0.09	0.61 ± 0.05	0.54 ± 0.09	0.43 ± 0.07
Spleen	0.45 ± 0.09	0.49 ± 0.13	0.35 ± 0.17	0.45 ± 0.08
Kidney	2.05 ± 0.31	2.34 ± 0.28	2.14 ± 0.39	2.51 ± 0.51
Liver	5.59 ± 1.29	5.81 ± 1.23	4.91 ± 1.41	6.09 ± 1.06
Blood	45.76 ± 10.48	36.85 ± 6.32	25.58 ± 3.59	19.75 ± 1.76
Muscle	25.13 ± 2.92	23.52 ± 1.56	22.86 ± 5.11	19.92 ± 2.97

...continued

TABLE XII (continued)

Tissue	Time After Administration		
	3 Hours	4 Hours	6 Hours
Bone	41.26 ± 10.45	43.01 ± 7.91	50.62 ± 6.23
Brain	0.14 ± 0.06	0.14 ± 0.06	0.21 ± 0.13
Lung	2.22 ± 0.67	2.19 ± 0.53	2.03 ± 0.46
G.I.T.	17.21 ± 2.92	17.23 ± 1.96	18.25 ± 5.93
Heart	0.54 ± 0.17	0.46 ± 0.22	0.29 ± 0.21
Spleen	0.49 ± 0.28	0.37 ± 0.17	0.48 ± 0.31
Kidney	2.02 ± 0.26	2.22 ± 0.27	2.27 ± 0.72
Liver	5.72 ± 1.23	5.43 ± 1.01	5.90 ± 0.99
Blood	18.74 ± 3.70	16.28 ± 1.17	16.12 ± 2.51
Muscle	21.36 ± 2.98	26.12 ± 6.62	20.72 ± 12.71

[a] Expressed as percent of injected radioactivity per whole organ. Bone, muscle and blood estimated to constitute 10%, 43% and 7% of total body weight, respectively.

[b] Mean of five animals ± standard deviation

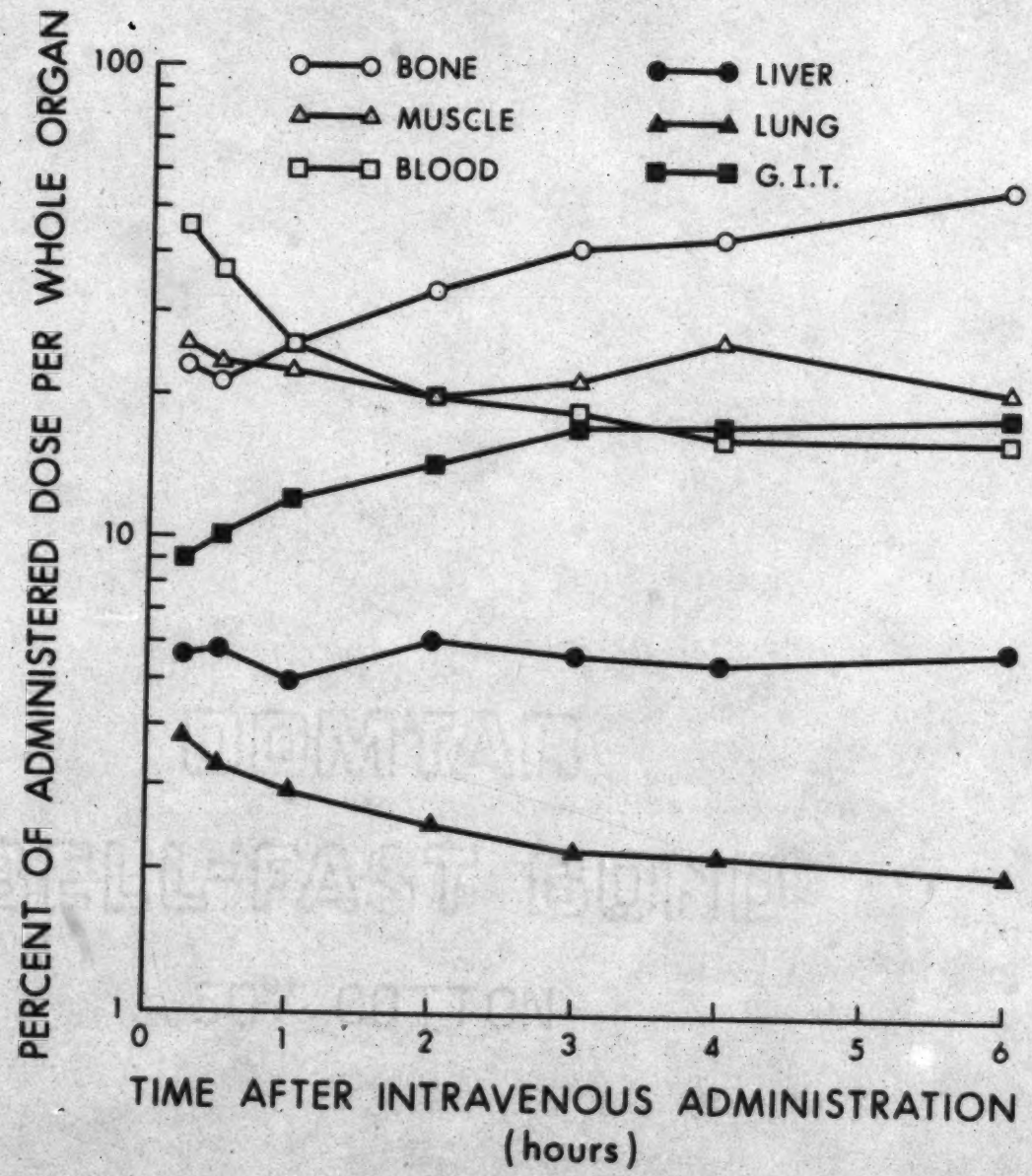

Figure 6

Whole Organ Uptake of ^{68}Ga-Polyphosphate in Mice

in the feces at six hours postinjection. The uptake of the complex in the G.I.T. at the same time was approximately 18% (Table XII). It has been reported that ^{67}Ga-citrate is normally taken up by the mucous membranes of the stomach and intestines (52). On the other hand, a polyphosphate such as ^{99m}Tc-STPP did not show any significant uptake in the G.I.T. (3). Thus, the uptake of the complex by the G.I.T. was considered to be due to the normal binding of ^{68}Ga to the mucous membranes of the stomach and intestines.

In animal studies using ^{51}Cr-polyphosphate a six hour post-injection muscle uptake of 3.23% of the injected dose per gram of muscle was reported (112). In the present study, ^{68}Ga-polyphosphate showed an uptake of 1.82% of the injected dose per gram of muscle after the same interval of time. A three hour postinjection study using ^{99m}Tc-STPP in rabbits reported an uptake of 4.69% of the injected dose by muscle (3), compared to an uptake of about 21% per total muscle of mice as determined in the experimental work. No reports in the literature were found indicating the expected normal uptake of gallium by muscle tissue.

Table XIII shows the concentration of radioactivity in each organ compared to the radioactivity in the blood at the various time intervals. From these results it is obvious that the bone tissue was the target organ, showing the highest ratio as compared to all other organs. In fact, the bone tissue concentrated over 50% of the injected dose

TABLE XIII

Tissue:Blood Ratio of Radioactivity in Various Organs After Intravenous Administration of ^{68}Ga-Polyphosphate[a,b]

Tissue	Time After Administration			
	15 Minutes	30 Minutes	1 Hour	2 Hours
Bone	0.35	0.40	0.70	1.18
Brain	0.04	0.03	0.02	0.03
Lung	0.75	0.81	1.01	0.89
G.I.T.	0.10	0.14	0.26	0.38
Heart	0.27	0.31	0.37	0.37
Spleen	0.23	0.28	0.32	0.36
Kidney	0.19	0.28	0.39	0.57
Liver	0.17	0.21	0.26	0.36
Muscle	0.09	0.10	0.14	0.16

...continued

TABLE XIII (continued)

Tissue	Time After Administration		
	3 Hours	4 Hours	6 Hours
Bone	1.52	1.84	2.19
Brain	0.03	0.04	0.06
Lung	1.06	0.98	0.92
G.I.T.	0.51	0.57	0.63
Heart	0.46	0.43	0.34
Spleen	0.46	0.44	0.64
Kidney	0.56	0.63	0.74
Liver	0.39	0.41	0.51
Muscle	0.18	0.26	0.21

[a] Relationship between the percent of injected radioactivity in one gram of tissue to that in one ml of blood

[b] Mean of five animals

six hours after administration of the ^{68}Ga-polyphosphate.

B. ^{68}Ga-Gallium-Polyphosphate Tissue Distribution Studies

The addition of carrier gallium to the ^{68}Ga-polyphosphate complex produced a very noticeable effect on the levels of radioactivity in both the lung and in the blood. The radioactivity in each of these tissues was approximately one third that noted for ^{68}Ga-polyphosphate after 15 minutes postinjection. The tissue distribution data (Table XIV) for the lung and blood are again simialr. The addition of carrier gallium to ^{68}Ga-citrate has been reported to cause saturation of the gallium binding proteins producing an enhanced skeletal uptake of the ^{68}Ga-citrate (36,41,84). As is shown in Table XIV and Figure 7, when the blood levels decreased, there was a corresponding increase in bone tissue uptake. It appeared that as the blood was cleared of the ^{68}Ga radioactivity, more ^{68}Ga then became available for uptake in the bone. Maximum bone uptake occurred after a postinjection period of about one hour compared to about three hours previously observed with ^{68}Ga-polyphosphate. The decreased blood levels were also reflected in an increased urinary excretion of radioactivity. After four hours, the cumulative urinary excretion was about 35% compared to about 6% in the case of ^{68}Ga-polyphosphate for the same time interval.

The concentration of radioactivity in the G.I.T.

TABLE XIV

Tissue Concentration of Radioactivity After Intravenous Administration of ^{68}Ga-Gallium-Polyphosphate in Mice[a,b]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	14.84 ± 1.73	21.70 ± 2.12	20.79 ± 3.33	23.62 ± 3.74
Brain	0.33 ± 0.24	0.16 ± 0.06	0.12 ± 0.06	0.12 ± 0.05
Lung	5.43 ± 0.73	3.38 ± 1.05	2.45 ± 0.55	2.42 ± 0.87
G.I.T.	1.00 ± 0.15	1.05 ± 0.14	1.28 ± 0.21	1.88 ± 0.25
Heart	2.14 ± 0.31	1.23 ± 0.32	0.92 ± 0.55	0.76 ± 0.88
Spleen	2.21 ± 0.27	1.24 ± 0.26	1.21 ± 0.29	0.95 ± 0.84
Kidney	6.18 ± 0.86	4.10 ± 0.47	3.17 ± 0.58	3.32 ± 0.69
Liver	2.89 ± 0.64	1.61 ± 0.27	1.62 ± 0.42	1.81 ± 0.42
Blood[c]	7.82 ± 1.35	3.67 ± 0.95	2.98 ± 1.16	3.15 ± 0.40
Muscle	1.04 ± 0.20	0.73 ± 0.28	0.59 ± 0.31	0.44 ± 0.24

[a] Percent of injected radioactivity per gram of tissue

[b] Mean of five animals ± standard deviation

[c] Percent of injected radioactivity per millilitre of blood

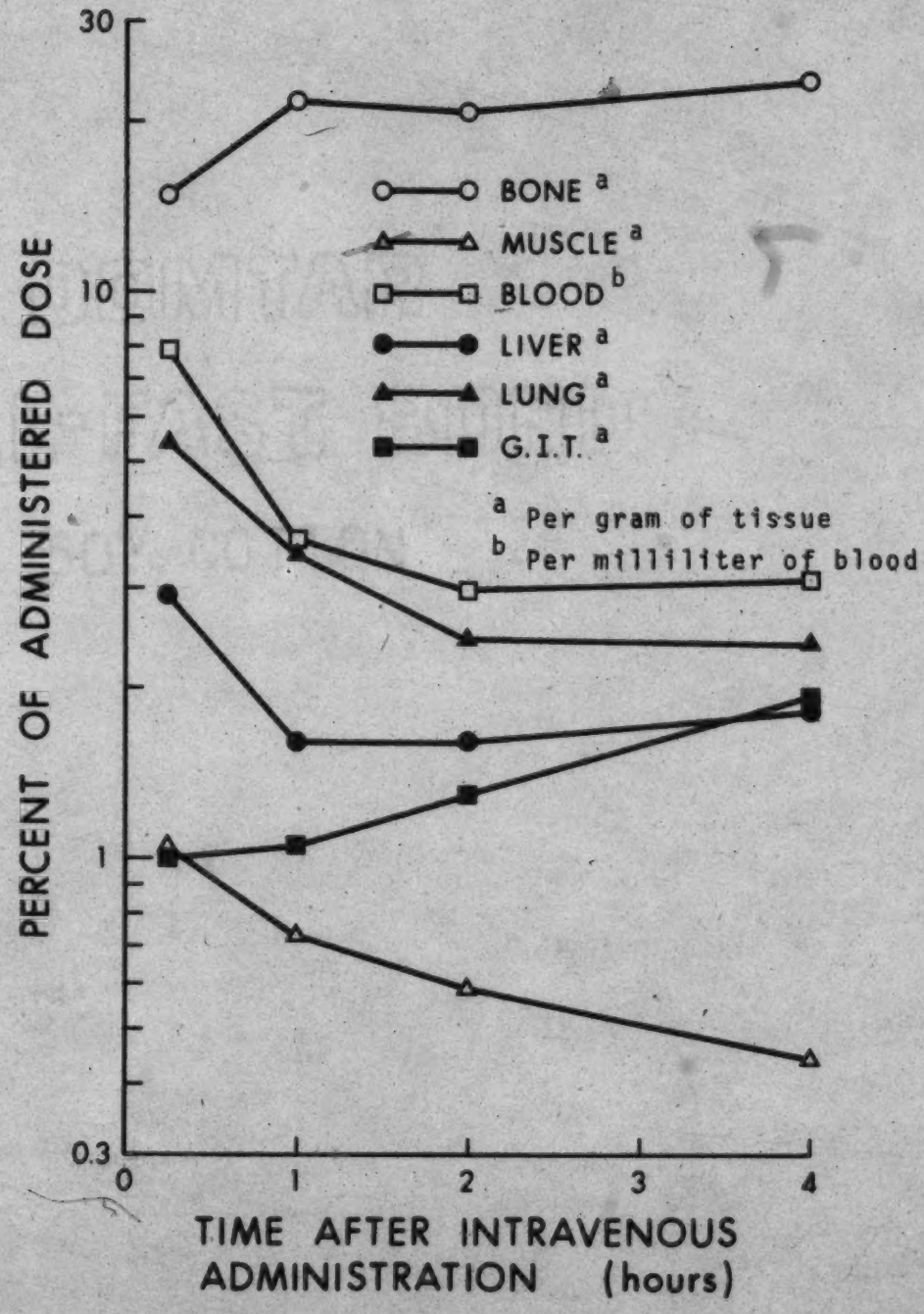

Figure 7

Tissue Distribution of ^{68}Ga-Gallium-Polyphosphate in Mice

and muscle was consequently lower when carrier gallium was added to the ^{68}Ga-polyphosphate. An interesting observation at this point was that the radioactivity in the feces did not increase as the level of radioactivity decreased in the G.I.T.. Whereas the level of radioactivity in the feces four hours postinjection for ^{68}Ga-polyphosphate was approximately 2.3%, that of ^{68}Ga-gallium-polyphosphate was approximately 0.5% of the injected dose.

Table XV and Figure 8 show that the whole organ uptake in the bone tissue reached 42% only 15 minutes after administration and attained a maximum level of 64% after four hours. The bone-to-blood ratio of 7.49 (Table XVI) after four hours also reflects this active uptake by the bone tissue. This value is considerable larger than that observed with ^{68}Ga-polyphosphate at the same time interval (Table XIII) and is approximately 10-15 times greater than any other tissue with the exception of the kidney. With ^{99m}Tc-STPP in the rabbit, the maximum bone-to-blood ratio of 6.1% was obtained only after a 24 hour postinjection period (3).

C. $^{68}Ga(OH)_3$ Tissue Distribution Studies

Since $^{68}Ga(OH)_3$ is known to exist partially in a colloidal form, it was expected that the liver, spleen and bone marrow would be the major organs of uptake. In fact, the liver, spleen and lungs were the organs that showed the

TABLE XV

Whole Organ Uptake of ^{68}Ga-Gallium-Polyphosphate After Intravenous Administration in Mice[a,b]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	42.41 ± 4.38	58.53 ± 5.27	56.36 ± 7.72	64.03 ± 8.55
Brain	0.14 ± 0.09	0.07 ± 0.02	0.05 ± 0.03	0.05 ± 0.02
Lung	1.27 ± 0.28	0.84 ± 0.30	0.52 ± 0.14	0.54 ± 0.19
G.I.T.	4.29 ± 0.85	3.72 ± 0.69	4.10 ± 0.91	5.49 ± 0.83
Heart	0.24 ± 0.02	0.14 ± 0.04	0.10 ± 0.05	0.09 ± 0.10
Spleen	0.23 ± 0.07	0.12 ± 0.03	0.13 ± 0.06	0.10 ± 0.09
Kidney	2.85 ± 0.64	1.67 ± 0.28	1.29 ± 0.32	1.27 ± 0.27
Liver	4.39 ± 0.95	2.36 ± 0.48	2.33 ± 0.36	2.68 ± 0.61
Blood	15.66 ± 2.76	6.94 ± 1.78	5.62 ± 2.00	6.03 ± 2.11
Muscle	12.76 ± 2.35	8.46 ± 3.24	6.81 ± 3.42	5.14 ± 2.93

[a] Expressed as percent of injected radioactivity per whole organ. Bone, muscle and blood estimated to constitute 10%, 43% and 7% of total body weight, respectively.

[b] Mean of five animals ± standard deviation

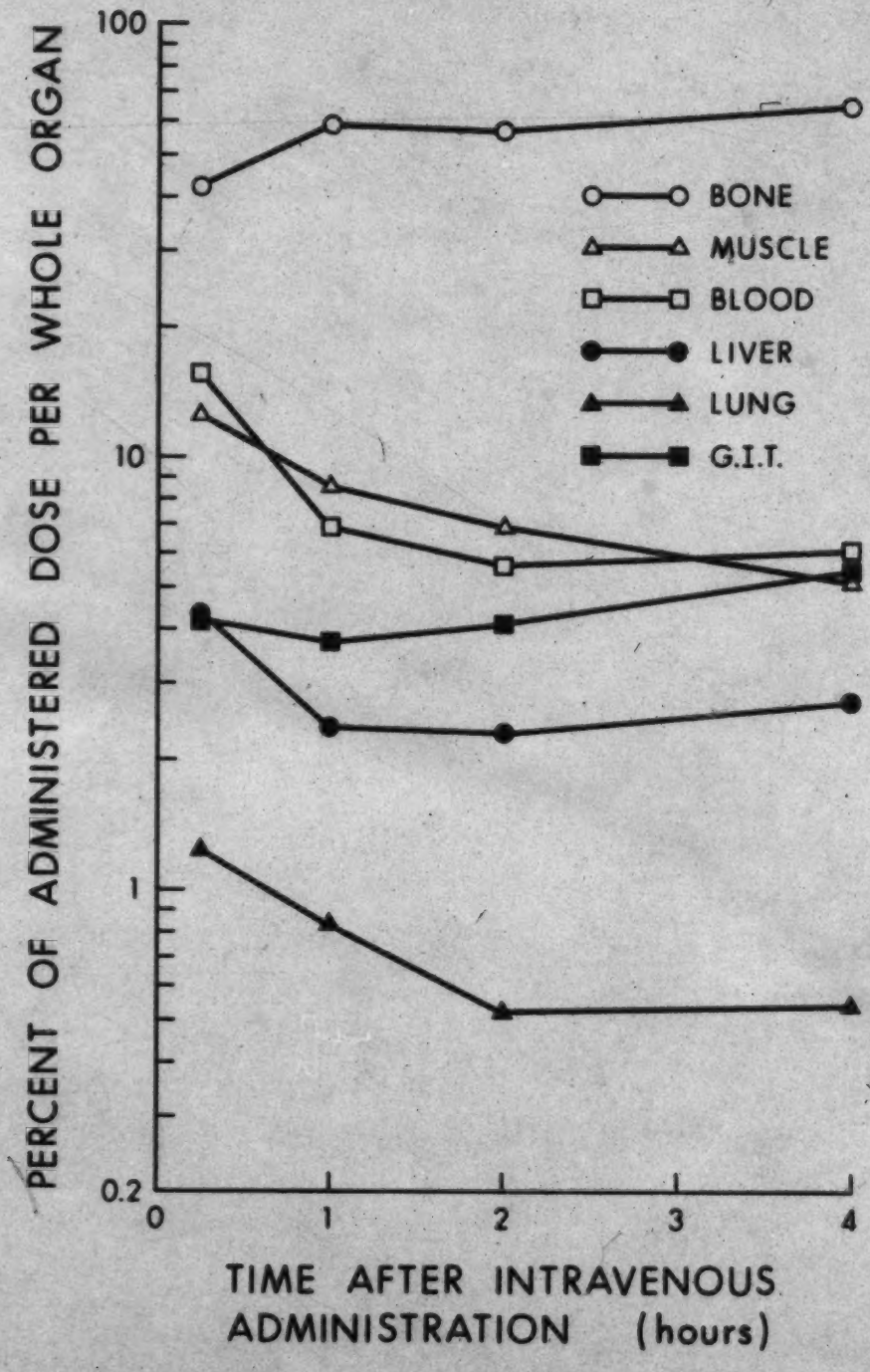

Figure 8

Whole Organ Uptake of ^{68}Ga-Gallium-Polyphosphate in Mice

TABLE XVI

Tissue:Blood Ratio of Radioactivity in Various Organs After Intravenous Administration of ^{68}Ga-Gallium-Polyphosphate[a,b]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	1.89	5.91	6.99	7.49
Brain	0.04	0.04	0.04	0.04
Lung	0.69	0.92	0.82	0.77
G.I.T.	0.13	0.29	0.43	0.59
Heart	0.27	0.34	0.31	0.24
Spleen	0.28	0.34	0.41	0.30
Kidney	0.79	1.12	1.07	1.05
Liver	0.37	0.44	0.55	0.57
Muscle	0.13	0.19	0.19	0.14

[a] Relationship between the percent of injected radioactivity in one gram of tissue to that in one ml of blood

[b] Mean of five animals

highest uptake (Table XVII) suggesting the formation of colloidal material. The radioactivity in the lungs and blood now displayed different distribution curves (Figure 9). The lung retained relatively more radioactivity than the blood, possibly indicating that larger particles had formed in the preparation which were trapped in the lung capillary bed.

Table XVIII and Figure 10 show that the liver concentrated 35% of the total administered dose within four hours after the injection, whereas the bone tissue localized only half that amount after the same time period. The tissue-to-blood ratio (Table XIX) showed that the liver, spleen and lungs were more active than the bone in removing the ^{68}Ga radioactivity from the blood. Other colloidal compounds such as ^{99m}Tc-sulfur colloid (135), ^{68}Ga-chromic phosphate colloid (88) and ^{68}Ga-hydrous ferric oxide colloid (85) have shown similar distribution patterns.

At this point it was not possible to ascertain if the radioactivity in the bone was due to an uptake of the ^{68}Ga preparations by the bone mineral itself or by the bone marrow. A separate experiment was therefore designed to further investigate this aspect using the rabbit as a model, the results of which are presented below.

III. Tissue Distribution in Rabbits

The results of the tissue distribution studies performed on mice indicated that the major site of uptake of

TABLE XVII

Concentration of Radioactivity After Intravenous Administration of ^{68}Ga-Hydroxide in Mice[a,b,c]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	5.44 ± 2.81	5.96 ± 0.53	6.29 ± 1.02	6.78 ± 1.69
Brain	0.35 ± 0.13	0.15 ± 0.10	0.26 ± 0.06	0.17 ± 0.09
Lung	14.79 ± 3.41	12.53 ± 2.31	9.08 ± 1.88	8.57 ± 2.70
G.I.T.	1.51 ± 0.35	2.31 ± 0.39	2.65 ± 0.57	2.41 ± 0.48
Heart	3.71 ± 1.07	1.68 ± 0.95	2.27 ± 0.63	1.74 ± 0.27
Spleen	15.27 ± 7.69	9.77 ± 4.16	9.61 ± 5.65	9.05 ± 2.06
Kidney	4.05 ± 1.42	3.12 ± 1.74	3.95 ± 0.82	2.91 ± 0.86
Liver	21.64 ± 3.82	19.32 ± 1.82	20.98 ± 1.29	21.93 ± 1.48
Blood[c]	12.76 ± 5.09	4.97 ± 3.97	6.61 ± 0.86	4.05 ± 1.13
Muscle	1.66 ± 0.54	1.42 ± 0.35	1.12 ± 0.36	0.87 ± 0.13

[a] Percent of injected radioactivity per gram of tissue

[b] Mean of five animals ± standard deviation

[c] Percent of injected radioactivity per millilitre of blood

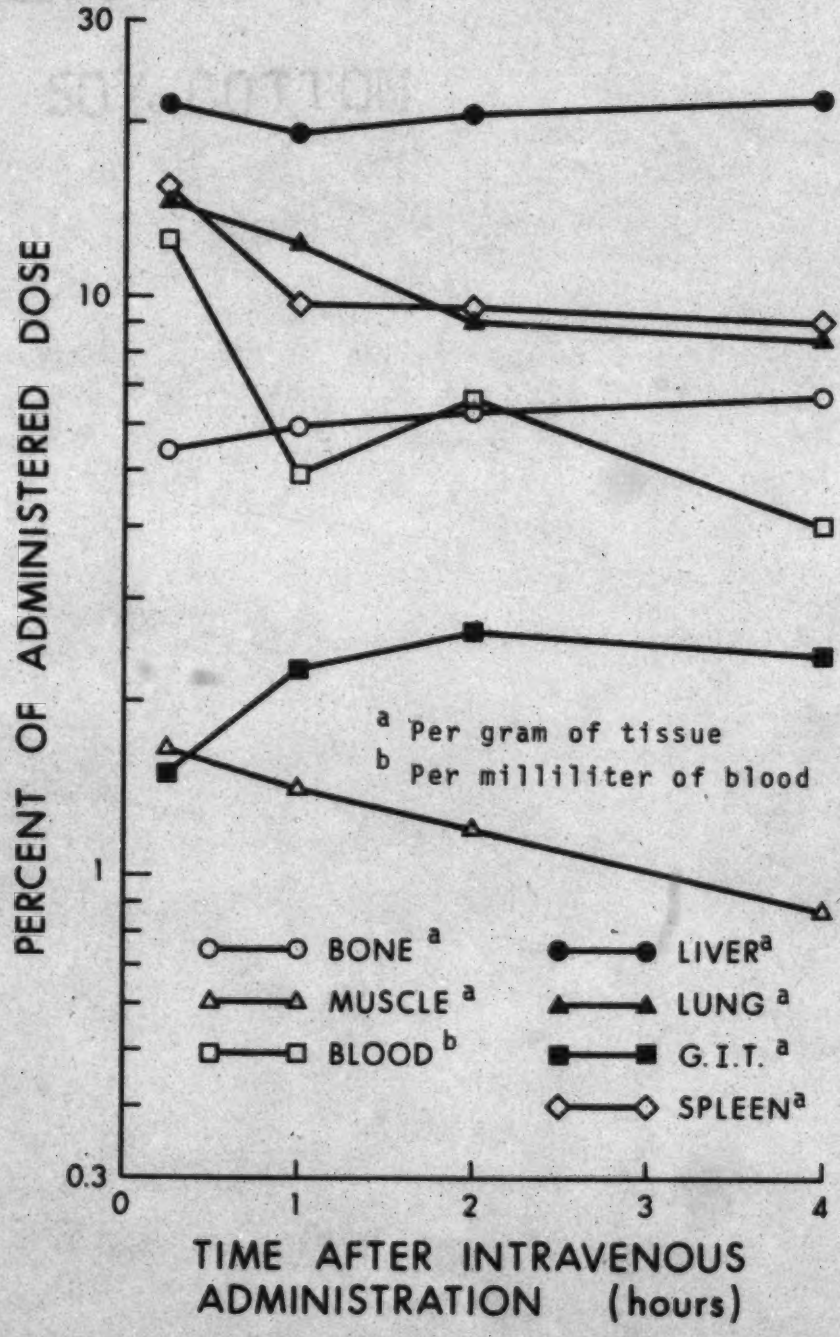

Figure 9

Tissue Distribution of ^{68}Ga-Hydroxide in Mice

TABLE XVIII

Whole Organ Uptake of ^{68}Ga-Hydroxide After Intravenous Administration in Mice[a,b]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	14.35 ± 6.41	17.24 ± 1.79	19.03 ± 3.43	18.84 ± 4.79
Brain	0.16 ± 0.05	0.07 ± 0.04	0.11 ± 0.03	0.07 ± 0.04
Lung	3.06 ± 0.29	2.71 ± 0.62	1.68 ± 0.31	1.87 ± 0.36
G.I.T.	5.64 ± 1.37	6.81 ± 0.53	8.26 ± 0.75	8.24 ± 1.53
Heart	0.40 ± 0.11	0.21 ± 0.15	0.30 ± 0.11	0.23 ± 0.06
Spleen	1.28 ± 0.44	0.76 ± 0.24	0.87 ± 0.33	0.74 ± 0.23
Kidney	1.77 ± 0.68	1.39 ± 0.77	1.74 ± 0.36	1.29 ± 0.41
Liver	32.22 ± 7.72	29.17 ± 2.82	31.73 ± 4.94	35.10 ± 3.79
Blood	24.32 ± 10.25	10.67 ± 9.27	14.04 ± 2.44	7.89 ± 2.36
Muscle	18.99 ± 4.73	17.39 ± 3.20	14.62 ± 5.29	10.38 ± 1.23

[a] Expressed as percent of injected radioactivity per whole organ. Bone, muscle and blood estimated to constitute 10%, 43% and 7% of total body weight, respectively.

[b] Mean of five animals ± standard deviation

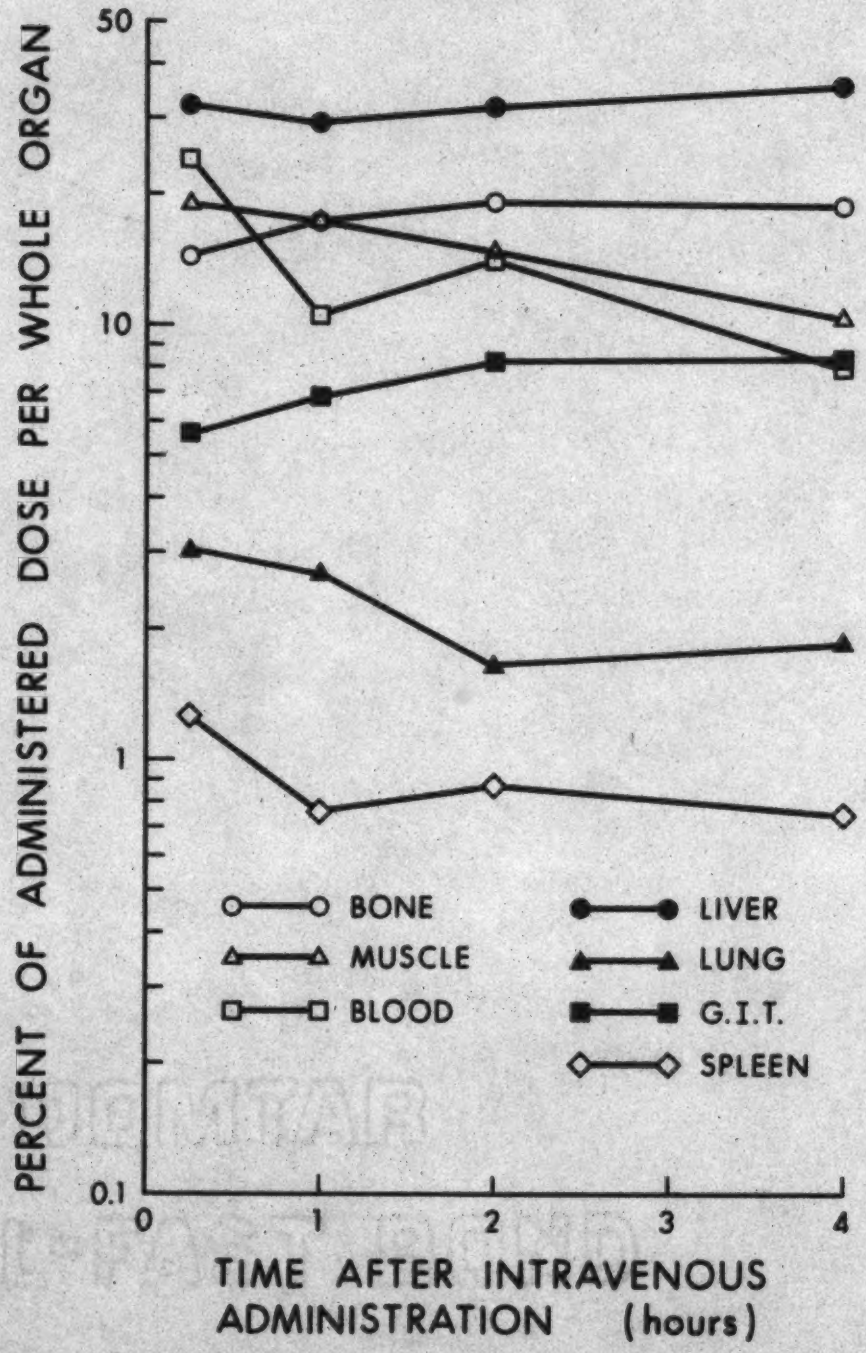

Figure 10

Whole Organ Uptake of ^{68}Ga-Hydroxide in Mice

TABLE XIX

Tissue:Blood Ratio of Radioactivity in Various Organs After Intravenous Administration of ^{68}Ga-Hydroxide[a,b]

Tissue	Time After Administration			
	15 Minutes	1 Hour	2 Hours	4 Hours
Bone	0.43	1.19	0.95	1.67
Brain	0.03	0.03	0.04	0.04
Lung	1.16	2.52	1.37	2.11
G.I.T.	0.12	0.47	0.40	0.59
Heart	0.29	0.34	0.34	0.43
Spleen	1.19	1.97	1.45	2.23
Kidney	0.32	0.63	0.59	0.72
Liver	1.69	3.89	3.17	5.41
Muscle	0.13	0.29	0.17	0.22

[a] Relationship between the percent of injected radioactivity in one gram of tissue to that in one ml of blood

[b] Mean of five animals

both ^{68}Ga-polyphosphate and ^{68}Ga-gallium-polyphosphate was in the bone and that the ^{68}Ga-gallium-polyphosphate complex had the greater bone-to-blood ratio. However, $^{68}Ga(OH)_3$ was primarily concentrated by the liver and spleen. Rabbit distribution studies were undertaken to determine:

(i) whether the $^{68}Ga(OH)_3$ was taken up in the bone marrow

(ii) to what extent, if any, the ^{68}Ga-gallium-polyphosphate complex accumulated in the bone marrow.

Table XX shows the results obtained for these two compounds in rabbits three hours after intravenous administration. The bone mineral concentrated the ^{68}Ga-gallium-polyphosphate almost 20 times more than did the bone marrow. Also, since the concentration of ^{68}Ga-gallium-polyphosphate in the liver was approximately 200 times less than for $^{68}Ga(OH)_3$, it was concluded that the ^{68}Ga-gallium-polyphosphate was essentially non-colloidal in nature. A bone mineral-to-bone marrow ratio of approximately three was obtained for the $^{68}Ga(OH)_3$. The spleen and liver were the only other tissues that showed a significant uptake of the $^{68}Ga(OH)_3$.

The bone-to-tissue ratios for the two ^{68}Ga-radiopharmaceuticals three hours after the intravenous administration to rabbits are shown in Table XXI. The bone-to-marrow, bone-to-muscle and bone-to-blood ratios reported

TABLE XX

Concentration of Radioactivity Three Hours After the Intravenous Administration of Various Gallium-68 Radiopharmaceuticals in Rabbits[a]

Tissue	Compound	
	^{68}Ga-Gallium-Polyphosphate	$Ga(OH)_3$
Bone Mineral	0.48	0.32
Bone Marrow	0.02	0.11
Liver	0.01	0.22
Lung	0.01	0.03
Spleen	0.06	1.01
Kidney	0.05	0.07
Muscle	0.003	0.01
Blood[b]	0.02	0.04

[a] Expressed as percent of injected radioactivity per gram of tissue.

[b] Expressed as percent of injected radioactivity per millilitre of blood.

TABLE XXI

Bone:Tissue Ratio After Intravenous Injection of Gallium-68 Radiopharmaceuticals in Rabbits[a]

Tissue	Compound			
	^{68}Ga-Gallium-Polyphosphate		^{68}Ga(OH)$_3$	
	Per Gram[b]	Per Total Organ[c]	Per Gram[b]	Per Total Organ[c]
Bone:Marrow	19.9:1	91.8:1	2.9:1	13.1:1
Bone:Muscle	159.7:1	37.1:1	29.0:1	6.8:1
Bone:Blood	23.4:1	33.4:1	8.6:1	12.3:1

[a] As measured three hours after intravenous administration.

[b] Relationship between the percent of injected radioactivity in one gram of bone to that in one gram of tissue.

[c] Relationship between the total radioactivity in bone to that of the total radioactivity in the various tissues. Bone, muscle, blood and marrow estimated to constitute 10%, 43%, 7% and 2.2% of the total body weight, respectively (3,120).

for ^{99m}Tc-STPP in rabbits at the same time interval were 9.9, 37 and 3.1 respectively (3). It is obvious that the highest bone-to-background ratio would be obtained for ^{68}Ga-gallium-polyphosphate when used for bone imaging in rabbits compared to either $^{68}Ga(OH)_3$ or ^{99m}Tc-STPP.

IV. Toxicity Studies

A. Acute Toxicity

The acute toxicity of sodium tripolyphosphate and of sodium tripolyphosphate containing carrier gallium was evaluated in mice after the intravenous administration of various dose levels contained in 0.25 ml. The results of these studies are presented in Table XXII and Table XXIII.

Only the 200 mg/kg dose of sodium tripolyphosphate resulted in any deaths. The deaths occurred rapidly, usually before the entire dose had been injected. The one surviving mouse at this dose level suffered muscle spasms which persisted for about one minute, after which the mouse recovered. Polyphosphates are known to cause a reduction in serum calcium levels (113, 114, 115, 116) which was probably the cause of death at this dosage. The acute toxicity of ^{99m}Tc-polyphosphate has been reported previously as being 150 mg/kg in mice (124).

No deaths occurred at any of the dose levels of sodium tripolyphosphate containing carrier gallium. A dose

TABLE XXII

Acute Toxicity of Sodium Tripolyphosphate After Intravenous Injection in Mice

Dose (mg/kg)	Number of Mice Injected	Number of Deaths
0.5	2	0
1.0	2	0
2.5	2	0
5.0	2	0
10.0	2	0
50.0	2	0
100.0	2	0
200.0	5	4

TABLE XXIII

Acute Toxicity of Sodium Tripolyphosphate Containing Carrier Gallium After Intravenous Injection in Mice

Dose (mg/kg)		Number of Mice Injected	Number of Deaths
Sodium Tripolyphosphate	Ga^{3+} as $GaCl_3$		
0.5	0.09	2	0
1.0	0.19	2	0
2.5	0.49	2	0
5.0	0.99	2	0
10.0	1.98	2	0
50.0	9.90	2	0
100.0	19.80	2	0

of 5 mg/kg of carrier gallium used in rat distribution studies previously (41) has been described as being objectionably high (42). However clinical studies using ^{68}Ga-citrate containing carrier gallium at a dose of 4 mg gallium/kg resulted in no apparent toxicity to the patients (36).

A polyphosphate dose of 0.5 mg/kg in the form of ^{99m}Tc-polyphosphate has been proposed for use in humans (123). Based on this dose level and on the results of the toxicity studies in mice, a ^{68}Ga-polyphosphate containing 0.5 mg polyphosphate /kg would provide a large safety factor for use in humans. Also, since a carrier dose of 4 mg gallium/kg has shown no apparent toxicity in humans (36), a ^{68}Ga-polyphosphate containing 0.5 mg polyphosphate and 0.25 mg gallium/kg would provide a fairly large margin of safety for use in humans.

B. Effect on Weight Gain

The surviving mice were observed over a 30 day period and their weights compared to those of control mice at various times. No appreciable differences between the weights of test and control mice over this period of time were observed. No long-term effects were grossly apparent.

C. Histopathological Studies

The results of the histopathological studies of the

liver, lung and kidney tissues excised at 7 and 30 days postinjection and for bone samples of rib, humerus and femur removed after 30 days showed no evidence of inflammatory, embolic, degenerative or any other significant changes. However, some specimens of lung exhibited artifactual changes due to the presence of large amounts of recently extravasated blood within the alveolar spaces, probably due to decapitation of the animals. Also, formalin pigment was found within certain tissues. It is known that formaldehyde absorbs ultraviolet light and is converted into formic acid unless a buffer is added to the formaldehyde solution. Since no buffer was added to the formaldehyde solution used, the presence of formalin pigment in the tissues was obviously due to the preservative.

V. Bone Images

A high bone-to-background ratio is a desirable characteristic of any bone imaging agent. This can be achieved by:

(i) a rapid uptake by bone of a large fraction of the administered radiopharmaceutical

(ii) an equally rapid clearance from surrounding tissue

(iii) a rapid urinary excretion of that portion of the dose not concentrated by the bone

Thus, a high bone-to-background ratio is reflected in high

bone-to-muscle and bone-to-blood ratios.

According to Tables XIII, XVI and XXIV, it was obvious that the ^{68}Ga-gallium-polyphosphate showed the highest bone-to-muscle and bone-to-blood ratios as compared to ^{68}Ga-polyphosphate. Whereas other investigators have reported bone-to-muscle and bone-to-blood ratios of 37:1 and 3:1 respectively for ^{99m}Tc-STPP (3) this present study indicated that ^{68}Ga-gallium-polyphosphate would yield bone-to-muscle and bone-to-blood ratios of approximately 160:1 and 23:1 respectively in rabbits after the same time period.

Based on these results, it appeared that the ^{68}Ga-gallium-polyphosphate complex had the highest bone-to-background ratio compared to either ^{68}Ga-polyphosphate or ^{99m}Tc-STPP. To further compare the utility of ^{68}Ga-polyphosphate and ^{68}Ga-gallium polyphosphate as potential bone imaging agents, the rabbit was used as a model, and bone images were obtained with the Pho/Gamma Positron III Camera. The results are presented in Figures 11 - 18. Figures 11 - 17 represent the hind quarter of a rabbit whereas Figure 18 displays the front section of a rabbit.

The best images for the ^{68}Ga-polyphosphate and for the ^{68}Ga-gallium-polyphosphate were obtained after a postinjection period of 120 and 145 minutes, respectively. However, these images do not necessarily represent maximum levels of radioactivity in the bone. Each image was

TABLE XXIV

Bone:Muscle Ratio of Radioactivity After the Intravenous Administration of Various Gallium-68 Radiopharmaceuticals in Mice[a,b]

Compound	Time After Administration						
	15 Minutes	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	6 Hours
^{68}Ga-Polyphosphate	4.01	3.85	4.89	7.27	8.23	7.12	10.47
^{68}Ga-Gallium-Polyphosphate	14.27	--	29.73	35.24	--	53.68	--
$^{68}Ga(OH)_3$	3.27	--	4.19	5.62	--	7.79	--

[a] Relationship between the percent of injected dose in one gram of bone to that in one gram of muscle

[b] Expressed as mean of five animals

Figure 11

Positron image obtained 30 minutes after the intravenous administration of ^{68}Ga-polyphosphate in a rabbit

Focal plane: 3 inches
Accumulated count: 18,000 counts

Figure 12

Positron image obtained one hour after the intravenous administration of ^{68}Ga-polyphosphate in a rabbit.

Focal plane: 3 inches
Accumulated count: 17,000 counts

Figure 11

Figure 12

Figure 13

Positron image obtained 90 minutes after the intravenous administration of ^{68}Ga-polyphosphate in a rabbit.

Focal plane: 3 inches
Accumulated count: 17,000 counts

Figure 14

Positron image obtained 120 minutes after the intravenous administration of ^{68}Ga-polyphosphate in a rabbit.

Focal plane: 2 inches
Accumulated count: 30,000 counts

Figure 13

Figure 14

Figure 15

Positron image obtained 120 minutes after the intravenous administration of ^{68}Ga-polyphosphate in a rabbit.

Focal plane: 4 inches
Accumulated count: 30,000 counts

Figure 16

Positron image obtained 125 minutes after the intravenous administration of ^{68}Ga-gallium-polyphosphate in a rabbit.

Focal plane: 3.5 inches
Accumulated count: 103,000 counts

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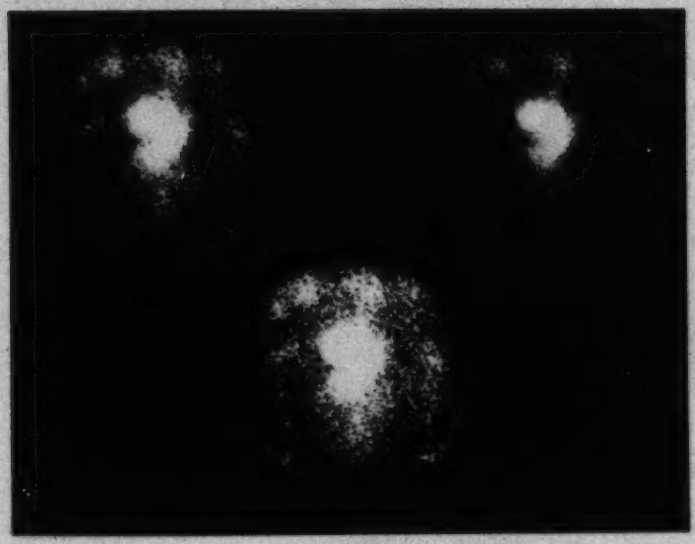

Figure 15

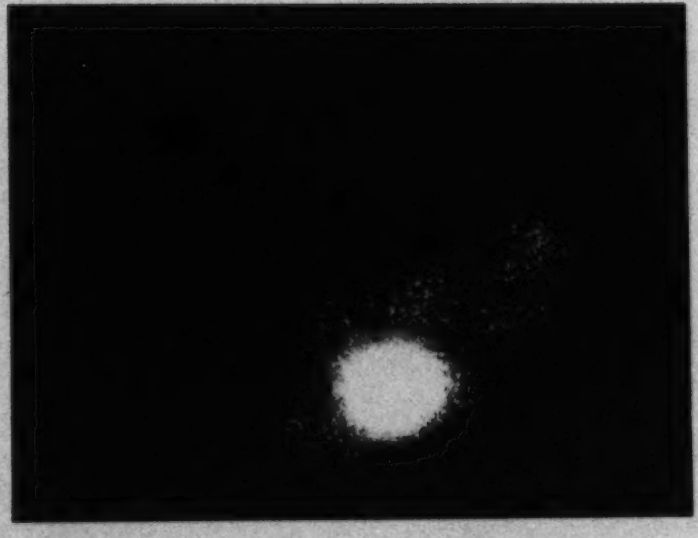

Figure 16

Figure 17

Positron image obtained 125 minutes after the intravenous administration of ^{68}Ga-gallium-polyphosphate in a rabbit.

Focal plane: 3 inches
Accumulated count: 103,000 counts

Figure 18

Positron image obtained 145 minutes after the intravenous administration of ^{68}Ga-gallium-polyphosphate in a rabbit.

Focal plane: 2.5 inches
Accumulated count: 25,000 counts

Figure 17

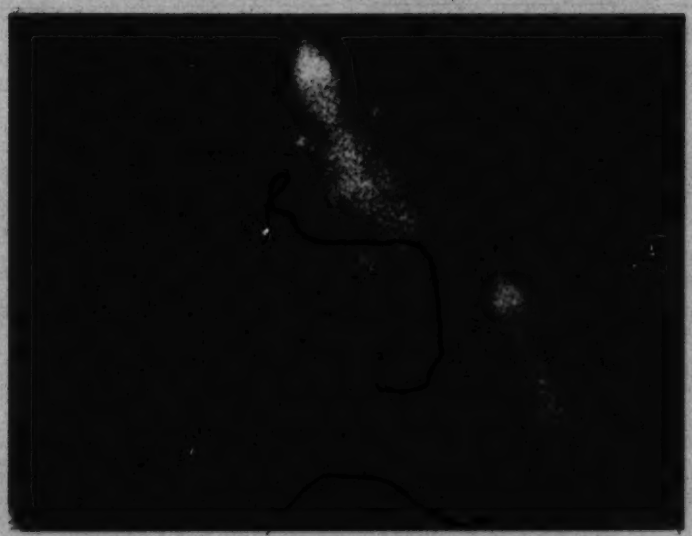

Figure 18

obtained using a certain focal plane setting which may not have been the best plane of focus. In order to detect a difference in the bone uptake characteristics of the two compounds it would be necessary to obtain additional images using various focal plane settings. Due to the low level of radioactivity injected (approximately 10 μCi) and the short half-life of the ^{68}Ga, it was not possible to obtain repeat images using different focal plane settings. Therefore, a valid comparison between these two agents would be difficult based on these few images obtained from two rabbits. These results, however, do indicate that both complexes can produce useful bone images within a 2 - 2.5 hour period. A commercial ^{99m}Tc-polyphosphate preparation currently used for bone imaging requires a delay of 3 - 4 hours before an imaging procedure can be started (124).

The large central "hot spot" as noted on most of the images was probably due to radioactivity in the bladder. As previously noted, the ^{68}Ga-polyphosphate and the ^{68}Ga-gallium-polyphosphate were excreted by mice to the extent of 6% and 35% respectively after four hours. This latter value is somewhat comparable to that reported by Subramanian (3) for ^{99m}Tc-STPP which was excreted to the extent of 45% following a three-hour distribution in the rabbit. In clinical practice, the radioactivity in the bladder could be minimized if the patient voided just prior to the scanning procedure.

Due to the short half-life of ^{68}Ga it was not possible to investigate the nature of any long-term effects which could have been produced by these polyphosphate complexes. Further work in this area using ^{67}Ga which has a 78 hour half-life may be useful. Recently, long-chained polyphosphates and other compounds such as the phosphonates have been investigated as ^{99m}Tc complexes for use as bone scanning agents (123,126). The possibility of forming such complexes with radioisotopes of gallium could be subjects for further investigation.

SUMMARY AND CONCLUSIONS

1) The dissociation of ^{68}Ga-EDTA was accomplished using 8N HCl. Separation of $^{68}Ga^{3+}$ from the EDTA was achieved with a Dowex 1-X4 anion exchange resin. The ^{68}Ga recovered was in the form of $^{68}GaCl_3$ which was later used in the preparation of ^{68}Ga-polyphosphate, ^{68}Ga-gallium-polyphosphate and $^{68}Ga(OH)_3$.

2) The preparation of ^{68}Ga-polyphosphate was based on the formation of a soluble complex using $^{68}GaCl_3$ in the presence of excess sodium tripolyphosphate. After the intravenous administration of this complex into mice, the major organs of uptake, 15 minutes after the injection, included the bone, blood, lung, G.I.T. and muscle. However, the concentration of radioactivity in the bone increased as the levels of radioactivity in the lungs and blood decreased. Six hours after administration the bone concentrated approximately 50% of the injected radioactivity.

3) The ^{68}Ga-gallium-polyphosphate complex attained maximum uptake in the bone approximately one hour after the administration to mice. The concentration of radioactivity in the blood and lungs was approximately one-third that noted for ^{68}Ga-polyphosphate after one hour. Bone-to-blood ratios for the two compounds indicated that the bone concentration of

the ^{68}Ga-gallium-polyphosphate was almost four times greater than that of ^{68}Ga-polyphosphate.

4) Rabbit tissue distribution studies using ^{68}Ga-gallium-polyphosphate yielded a bone-to-bone marrow ratio of 20:1, indicating that the complex was primarily deposited in the bone mineral itself, rather than the bone marrow.

5) The tissue distribution of $^{68}Ga(OH)_3$ in mice showed that the liver, spleen and lungs were the major organs of uptake, with the liver accumulating approximately 35% of the administered dose after four hours. Following the intravenous injection of $^{68}Ga(OH)_3$ into rabbits, the bone-to-bone marrow ratio was only 3:1.

6) Sodium tripolyphosphate, at a dose of 200 mg per kg, when injected into mice, produced death in four out of five animals. However, lower doses of the sodium tripolyphosphate in combination with various concentrations of carrier gallium appeared to be non-toxic. Examination of tissue slices from samples of lung, kidney, liver and bone (rib, humerus, femur) did not reveal any histopathological changes.

7) A comparison of the bone-to-muscle ratios of ^{68}Ga-polyphosphate and ^{68}Ga-gallium-polyphosphate four hours after intravenous administration to mice indicated that addition of carrier gallium increased the bone-to-muscle ratio by a factor of approximately 7. A similar comparison of the bone-to-blood ratios of these two compounds indicated that the ^{68}Ga-gallium-polyphosphate was cleared from the blood and deposited in the bone more rapidly than the ^{68}Ga-polyphosphate.

8) Bone images of rabbits obtained on a Pho/Gamma Positron III Camera illustrated that the bone concentrated the ^{68}Ga radioactivity after the intravenous injection of ^{68}Ga-polyphosphate or ^{68}Ga-gallium-polyphosphate. Useful images were obtained within two hours after the administration of the compounds.

9) Based on the above summary of results, it was concluded that:

   a) of the three ^{68}Ga radiopharmaceuticals investigated, the ^{68}Ga-gallium-polyphosphate complex demonstrated the highest level of accumulation in the bone of test animals;

   b) the ^{68}Ga-gallium-polyphosphate fulfils many of the desired characteristics of a useful bone scanning agent.

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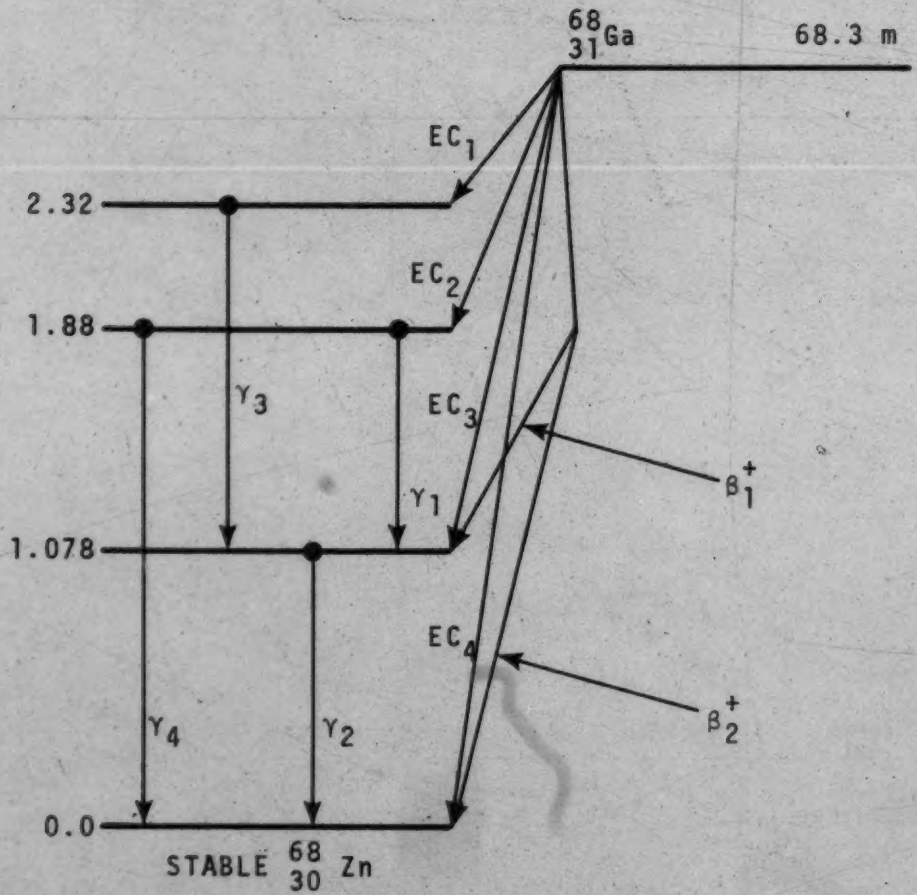
APPENDIX 1

Decay Scheme of ^{68}Ga (78)



APPENDIX 2

STATISTICAL EQUATIONS

EQUATION 1

Equation 1 and Computer Program A were used for calculating the level of ^{68}Ge contamination in the ^{68}Ge-^{68}Ga generator eluate.

$$\frac{A \pm a}{B \pm b} = \frac{A}{B} \pm \frac{A}{B} \sqrt{\frac{a^2}{A^2} + \frac{b^2}{B^2}}$$

where: A = Counts per minute due to the parent radioactivity at time of elution;

B = Counts per minute arising from ^{68}Ga at the time of elution;

a = Standard deviation of the parent count rate;

b = Standard deviation of the ^{68}Ga count rate.

EQUATION 2

The net count rate for each of the four samples of fraction B was calculated using Equation 2.

$$\bar{X} - \bar{Y} \pm \sqrt{\left(\frac{\sigma\bar{X}}{\sqrt{N}}\right)^2 + \left(\frac{\sigma\bar{Y}}{\sqrt{N}}\right)^2}$$

where: $\bar{X}$ = Mean sample count rate;

$\bar{Y}$ = Mean background count rate;

$\sigma\bar{X}$ = Standard deviation of mean sample count rate;

$\sigma\bar{Y}$ = Standard deviation of mean background count rate;

N = Total number of repetitions.

EQUATION 3

After the net count rate for each of the four samples of fraction B was calculated, the average net count rate of the four samples was determined using Equation 3.

$$\frac{\Sigma \overline{X}_1}{N} \pm \sqrt{(\sigma \overline{X}_1)^2 + (\sigma \overline{X}_2)^2 + (\sigma \overline{X}_3)^2 + (\sigma \overline{X}_4)^2}$$

where: $\frac{\Sigma \overline{X}_1}{N}$ = Average net count rate;

$(\sigma \overline{X}_{1-4})^2$ = Standard deviation of the mean net count rate of each sample;

N = Number of samples.

APPENDIX 3

COMPUTER PROGRAMS

COMPUTER PROGRAM A

Computer Program A in conjunction with Equation 1 was used for calculating the level of ^{68}Ge-contamination in the ^{68}Ge-^{68}Ga generator eluate. The data, as plotted in Figure 4, was obtained using Program A.

```
*C-FOCAL,69CE
*
*01.01 E
*01.10 A !!"DAUGHTER HALF LIFE",KB
*01.20 S KB=FLOG(2)/KB
*01.30 A !"DELAY BETWEEN PAIRS OF COUNTS "D
*01.45 A !"PLOT FUNCTION FOR TIME "LL," TO "LU," IN STEPS OF"LI
*01.47 T !!"   TIME        COUNTS"
*01.50 G 9.1
*
*03.10 S LA=SG*SA-SD*SC;S LB=SB*SD-SC*SG;
*03.20 S LC=SB*SA-SC'@;S LD=SB*SH-SG'2
*03.30 S SY=FSQT((LC*LD-LB'2)/(LC*SB*(N-2)))
*03.40 S SP=SY/(FSQT(LC/SB))
*03.50 S SQ=SY*(FSQT(1/SB+(SB*(SC/SB-TS)'2/LC))
*03.60 T !!"PATENT   '%,LA/LC,"   +OR- ",SQ
*03.65 T !"DAUGHTER ",LB/LC,"   +OR- ",SP,!!
*03.70 F I=LL,LI,LU;D 5
*03.80 T !!!;Q
*
*05.10 S TS=FEXP(-I*KB);S Y=LA/LC+LB*TS/LC;D 3.5
*05.20 T !"TIME ",I," COUNTS ",Y," +OR- ",SQ
*
*08.10 S X=FEXP(-KB*T)
*08.30 S SA=SA+W*X'2;S SB=SB+W;S SC=SC+W*X;
*08.40 S SD=SD+W*X*Y;S SG=SG+W*Y;S SH=SH+W*Y'2;S N=N+1;
*
*09.10 P;A SN;P;I (SN)3.1;P;A SN,Y,MA;P;S MA=MA/100
*09.20 I (MA)9.1,9.1;S T=MT-FLOG((1-FEXP(-KB*MA))/KB*MA)/KB
*09.25 P;A SN,SN,B,MB;P;S MB=MB/100;
*09.26 S W=1/(Y/MA'2+B/MB'2); S Y=Y/MA-B/MB;
*09.30 S MT=MT+MA+MB+D;T !%7.02,T,'   "%8.02,Y;D 8;G 9.1


DAUGHTER HALF LIFE:  68.3 Min
DELAY BETWEEN PAIRS OF COUNTS:  3.4 Min
PLOT FUNCTION FOR TIME:  0   TO: 1440  IN STEPS OF: 120
```

COMPUTER PROGRAM B

The following program was utilized for calculating the cumulative radioactivity in each portion of the ^{68}Ge-^{68}Ga generator eluate contained in a series of tubes (Table IX). The volume of eluate collected in each tube was estimated from the weight of the eluate and converted to volume, assuming a density of 1.0.

```
*C-FOCAL,69CE
*
*01.01 E
*01.10 T !!"TIME IN MINUTES   MASS IN GRAMS
VOLUME IN MLS";X
*01.20 A !!"NO OF SAMPLES"N,"   COLLECTION TIME"T
*01.30 A !"VOLUME COUNTED"D,"   % COUNTING EFFICIENCY"H,!!
*01.35 F I=1,N;D 3
*01.40 T !!"SAMPLE      SAMPLE  TOTAL  SPECIFIC  TOTAL"
*01.50 T !"NUMBER  TIME VOLUME VOLUME  ACTIVITY ACTIVITY"
*01.60 F I=1,N;S P=P+V(I);S Q=Q+V(I)*A(I);D 2
*01.70 T !!!!;X;Q
*
*02.10 T !"  "%2,I,"      "%4.02,I*T,"   ",V(I),"    ",P
*02.20 T "    "%8.06,A(I),"   ",Q
*
*03.10 T !%2,I,"  "
*03.20 A "EMPTY "X," FULL "Y," CPM "C," DELAY TIME "B,"
OK?"Z
*03.30 I (Z)3.1;S V(I)=Y-X;
*03.40 S A(I)=100*C*FEXP(B*FLOG(2)/68.3)/(D*H*2.22E+06)
**
*
*GO

TIME IN MINUTES   MASS IN GRAMS   VOLUME IN MLS

NO OF SAMPLES: 25    COLLECTION TIME: 30 Sec
VOLUME COUNTED: .01    % COUNTING EFFICIENCY: 21%
```

COMPUTER PROGRAM C

This program was used initially to calculate the radioactivity in each 1 ml portion of Dowex 1-X4 resin eluate and to calculate the percentage of the total radioactivity applied on the resin that was recovered in each 1 ml portion of eluate.

In animal distribution studies, Program C was used to calculate the radioactivity in each tissue and the percentage of the total administered radioactivity recovered in each tissue.

```
*E A
*
*C-FOCAL,69CE
*
*01.01 E
*01.10 T !!!"     *** TOTAL ELUTED ACTIVITY CALCULATIONS ***"
*01.15 A !!"VOL COUNTED IN MLS "V;
*01.17 A "DELAY IN MINS FROM END OF ELUTION "T
*01.20 A !"CPM OBSERVED "C,"BACKGROUND "B,"TOTAL ELUTED VOL "
*01.25 A "IN MLS "VT,!"% COUNTING EFFICIENCY "G,"
HALF-LIFE IN MINS "L
*01.30 S L=FLOG(2)/L;S X=100*VT*(C-B)/(G*V*2.22E+06);D
9;S AT=X
*01.35 T !!"TOTAL ACTIVITY "%,AT,"
MICRO-CURIES AT END OF ELUTION"
*01.40 T !!"COLUMN OUTPUT CALCULATIONS"!!"
TIME DELAY IN MINS BEFORE"
*01.45 A " FIRST COLUMN SAMPLE "TD,!"TIME DELAY BETWEEN COUNTING"
*01.50 A " SAMPLES "TX;T !!"                    TIME       "
*01.55 T "ACTIVITY       % OF TOTAL"
*01.60 T !"                  MINS     MICRO-CURIES      "
```

...continued

```
*
*02.10 D 8;S T=TD+TQ/2;S X=100*(C-B)/(G*2.22E+06);D 9;
*02.12 X;T !%7,C,"  ",%5,100*TQ,"  ";X
*02.15 T %5.02,T,"  "%,X,"  ",100*X/AT;S  TD=TD+TQ+TX;
*02.20 G 2.1
*
*08.10 P;A XX;P;I (XX)8.3;P;A XX,C,TQ;S TQ=TQ/100;P;I
(c)8.2,8.2,8.25
*08.20 S C=1000000
*08.25 S C=C/TQ;R
*08.30 T !!"ACTIVITIES CORRECTED FOR DECAY
TO END OF ELUTION PERIOD";Q
*
*09.10 S X=X*FEXP(L*T)
**
*
*GO


     *** TOTAL ELUTED ACTIVITY CALCULATIONS ***

VOL COUNTED IN MLS: 0   DELAY IN MINS FROM END OF ELUTION: 0
CPM OBSERVED: 0    BACKGROUND: 0    TOTAL ELUTED VOL IN MLS: 0
% COUNTING EFFICIENCY: 21    HALF-LIFE IN MINS: 68.3
*GO
```

COMPUTER PROGRAM D

The mean and standard deviation values for the data obtained in the tissue distribution studies at each time interval were calculated using Program D.

```
*E A
*
*C-FOCAL,69CE
*
*01.01 E
*01.02 T !!!"FOLLOW FIRST COLON ON A LINE BY DATA"
*01.03 T !"FOLLOW TEST :- BY A ZERO IF DATA OK; A ONE IF NOT;"
*01.04 T "A -1 AT END OF DATA"!!"INPUT"!"DATA"!
*01.10 A !X," Test",T
*01.20 I (-T) 1.65 ;S I=I+1;S A=A+S;S B=B+X*X;I (T) 1.6;G 1.1
*01.60 S M=A/I;S D=FSQT((B-A*A/I)/(I-1));T
!!"NUMBER OF DATA "%4,I;
*01.61 T !!"MEAN OF DATA "%,M,!!"STANDARD DEVIATION ",D;
*01.62 T !!"STANDARD ERROR OF THE MEAN ",D/ESQT(I),!!!;Q
*01.65 T "ERROR NOTED , DATA TO THE LEFT IGNORED -
TRY AGAIN";G 1.1
**
```